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COMPARATIVE SHORTENING VALUE OF SOME COMMERCIAL FATS

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Differences in the shortening power of fats have attracted much attention and numerous studies have been made of various shortening agents when incorporated into pastries, eggless cookies, and cakes. Lard has received the greatest emphasis and several reports are recorded on special lards, the physical and chemical properties of which were affected either by the feed of the animal, the type of fat from which the lard was made, or the method of manufacture. A study by Lowe, Nelson, and Buchanan (1938) includes, in addition to lards, many of the other fats that have become available to housewives in recent years.

Lowe (1937) states, "The degree of shortening produced by a fat or oil in a given product depends primarily upon the surface area of the flour particles covered by the fat." Attempts to relate the degree of shortening to some fundamental property of the fat have not proved very successful. Fisher (1933) found that the shortening value of a number of lards decreased as the congealing point increased but this was no longer true when hydrogenated fats and compounds were included. Following the work of Langmuir (1917) and Harkins, Clark, and Roberts (1920), showing that unsaturated fatty acids have greater covering power than saturated fatty acids, it was believed that differences in the shortening value of various fats might be explained by their degree of unsaturation. Lowe *et al.* (1938) found some correlation between the breaking strengths of pastries and the iodine numbers of the fats used. Again, this relationship was most evident for the lards. The data for a variety of fats showed that the degree of unsaturation was not the only factor influencing shortening value. In an earlier study Platt and Fleming (1923) had predicted that the iodine value could not be a criterion of shortening value because of the fact that linoleic acid containing two double bonds has no greater covering power than oleic acid with only one double bond. They believed that shortening power might depend upon the unsaturated glyceride content of a fat and presented some evidence to support this view.

A factor thought to influence the shortening power of fats is "plasticity," although this is a property that has never been measured quantitatively nor has it even been defined adequately. As nearly as can

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be determined, it seems to correspond to "consistency" or resistance to deformation. Harvey (1937) stated that increasing the plasticity of a fat by some means, such as mechanical manipulation or the addition of an edible oil, increased its shortening power. Lowe *et al.* (1938) also found that the addition of oleic acid to lards resulted in the formation of a more tender pastry. These investigators believed their results to be due to the increased number of double bonds present and to the reaction of free fatty acids with gluten rather than to a change in plasticity.

The object of the present investigation was to study in pastry and modified shortbread the shortening value of representative fats. Since fats are subjected to mechanical working during the process of being incorporated into dough, a study was made of the consistency of the fats under similar conditions in order to determine what effect this factor might have upon shortening value. Changes in the specific gravity of the fats during creaming were measured to see whether a relationship existed between the creaming volumes of the fats and their shortening power in shortbreads. The iodine number, free fatty acid, melting point, and congealing point of each fat were also determined.

EXPERIMENTAL PROCEDURE

The fats used in the study were commercial products available to the homemaker on the retail market. They included two samples of butter; an oleomargarine made from animal fat and one made from vegetable oils; three lards—a refined steam-rendered lard, a leaf lard, and a hydrogenated lard; an oleo-stearine cottonseed oil compound; and three brands of hydrogenated vegetable oil. The butter oil separated from Sample 2 of butter was also studied. According to the information that we were able to obtain, the hydrogenated fats were of the following composition: Sample 1, a partially hydrogenated cottonseed oil; Sample 2, a mixture of vegetable oils, largely cottonseed, partially hydrogenated; and Sample 3, a small part of fully hydrogenated cottonseed oil combined with a much larger proportion of the oil. This type of product is also called "hardened" fat.

To insure freshness of the fats each was obtained in retail packages from a local warehouse or, in some cases, directly from the manufacturer at the time the study of the fat began. Those fats recommended by the manufacturer to be kept at room temperature were stored in a room having a constant temperature of 22°C.(71.6°F.). The others were stored in a refrigerator at 4 to 7°C.(39.2 to 44.6°F.) and removed to the constant temperature room 24 hours before they were used in the experiment.

Immediately upon receipt of each fat the iodine number, per cent of free fatty acid, melting point, and congealing point were determined.

CONSISTENCY

Fats are plastic solids which exhibit thixotropic behavior; that is, they change progressively in consistency as a result of manipulation or working at a constant temperature. In order to measure the consistency of fats and to determine quantitatively the effect of manipulation, a procedure

was adapted from the "Tentative Method of Test for Penetration of Greases and Petrolatum" of the A.S.T.M. (1933). A universal precision penetrometer was used with the cone-shaped attachment made of aluminum to decrease its weight. The penetration of the weighted cone into the fat during one minute, measured in tenths of millimeters, was considered the consistency reading. The total weight exerted on the cone (including the weight of the cone itself) was 400 grams except that in some cases when the fat became very soft the weight was reduced to 200 grams. The fat was contained in metal rings of known volume, three and one-half inches in diameter and two and one-half inches deep, supported by a flat metal plate.

Whenever possible, readings were made on the undisturbed fat. The rings were filled by being pushed down through the center of the fat in the original container. In all cases except when the fat was marketed in pound prints, a ring was completely filled. Excess fat was cut away from the outside of the ring and the two surfaces leveled off with a spatula. This method of handling produced a minimum amount of disturbance in the fat. Two or three readings were taken on each surface of a ring of fat, care being taken to have the areas far enough apart that a test would not be affected by a previously disturbed area. The consistency was studied at 18, 22, and 26.5°C. (64.4, 71.6, and 79.7°F.). Determinations were made on samples from five containers at 18°C. and on two each at the other temperatures. An average was obtained from all of the readings at a given temperature.

After readings were completed on the undisturbed fat it was removed from the ring and cut with a grating of fine wires placed about one-tenth of an inch apart. The fat was cut through the grating four times and then packed into the ring with as little manipulation as possible, taking care to avoid the formation of air pockets. The consistency reading was taken, the fat was cut four more times, and another reading was taken. This process was continued until further disturbance of the fat produced little change in the reading or until the capacity of the penetrometer was reached. After being cut only a few times, some of the fats became very soft. In order to study the changing consistency in these instances, the weight on the cone was reduced to 200 grams.

The consistency curves for the fats cut at 18, 22, and 26.5°C. (Figs. 1, 2, and 3) show that in all cases there was a change in consistency as the fats were cut. This change was pronounced in all of the hydrogenated fats and less marked in the other fats. Since there was no relationship between the consistency of the undisturbed fat and the consistency after manipulation, it may be said that it is impossible to judge from the consistency of a fat in its container what the consistency will become as the fat goes through the process of being incorporated into pastry.

In general the effect of raising the temperature of the fats was to shift the curves upward. The amount that a curve was shifted varied, being least for the lards and hydrogenated fats and greatest for butters and oleomargarines. The butterfat curves occupied the lowest positions of the fats studied at 18°C. At 22°C. there was only one curve above them and

at 26.5°C. only one of the butterfats was firm enough to be studied in this way.

MEASUREMENT OF SHORTENING VALUE

Pastries: Pastries contain a high percentage of fat, and the fat is combined with the other ingredients with a minimum of mixing. The formulas used for preparing the pastries were as follows:

	100-per cent fats	80-per cent fats
Flour (all-purpose or family).................	200 gm.	200 gm.
Fat..	82 gm.	103 gm.
Water...	45 ml.	35 ml.
Salt..	4 gm.	......

When butter and margarines were used the formula was adjusted so that the quantity of fat, water, and salt would correspond as nearly as possible

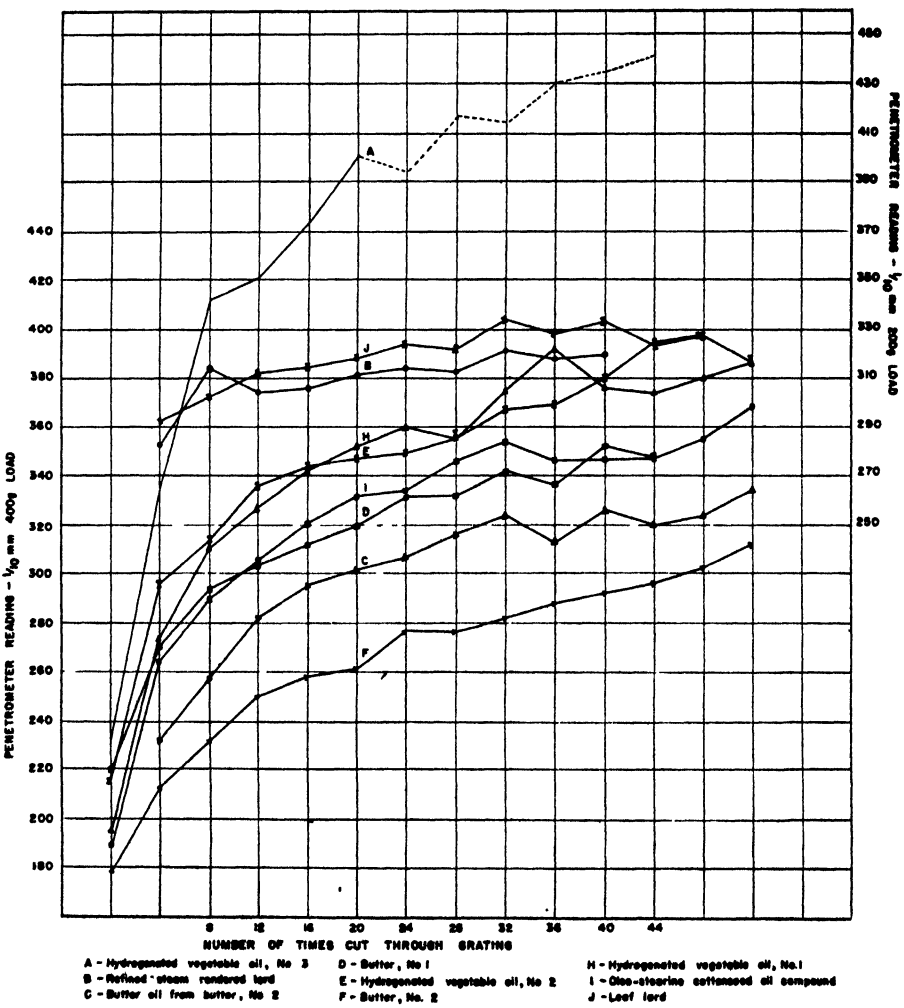

FIG. 1. Consistency of fats as affected by mechanical working at 18°C.(64.4°F.). Dotted lines represent readings taken with a 200-gram load.

to that present when 100-per cent fats were used. The quantity of water could not be made to correspond exactly, however, because all of the water present in the 80-per cent fats is not available for wetting purposes.

The dough was prepared in a constant temperature and humidity room. Flour, salt, and fat were mixed together at low speed for five minutes in the 10-quart container of a mixer using the flat beater. The water was added during five seconds of mixing at the same speed, and the mixing was continued for 55 seconds. The dough was shaped with six motions of the hand. It was then divided in halves, and each half was rolled between sheets of parchment paper to a uniform thickness of one-eighth inch with the aid of a metal frame as a guard. A specially made cutter was used to cut the pastry into pieces one and three-eighths by two and

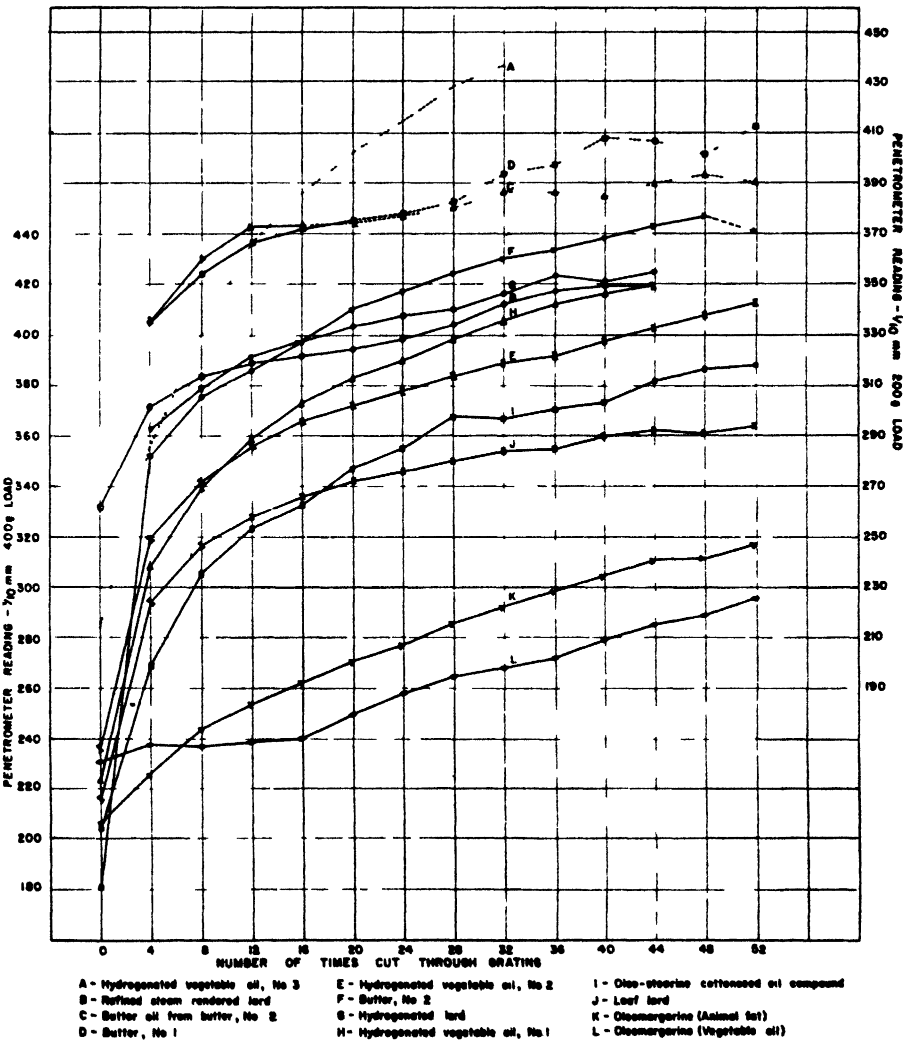

FIG. 2. Consistency of fats as affected by mechanical working at 22°C.(71.6°F.). Dotted lines represent readings taken with a 200 gram load.

one-eighth inches. Heavy metal strips of proper dimensions, covered with cellophane to prevent sticking, were placed on the frame and used as cutting guides. Ten pastries (two rows of five each) were cut from each half of the dough and arranged on a perforated baking sheet in the same order as they appeared in the metal frame. The sheet, containing 20 pastries, was transferred immediately to a large electric oven with a brick hearth and placed on a rack about one inch above the hearth. The pastries were baked for 13 minutes at 218°C.(424.4°F.), removed to the constant temperature room, allowed to cool for 15 minutes, and broken on the Bailey (1934) shortometer.

Breaking strengths were obtained for pastries made from doughs mixed under the following conditions of temperature and relative humid-

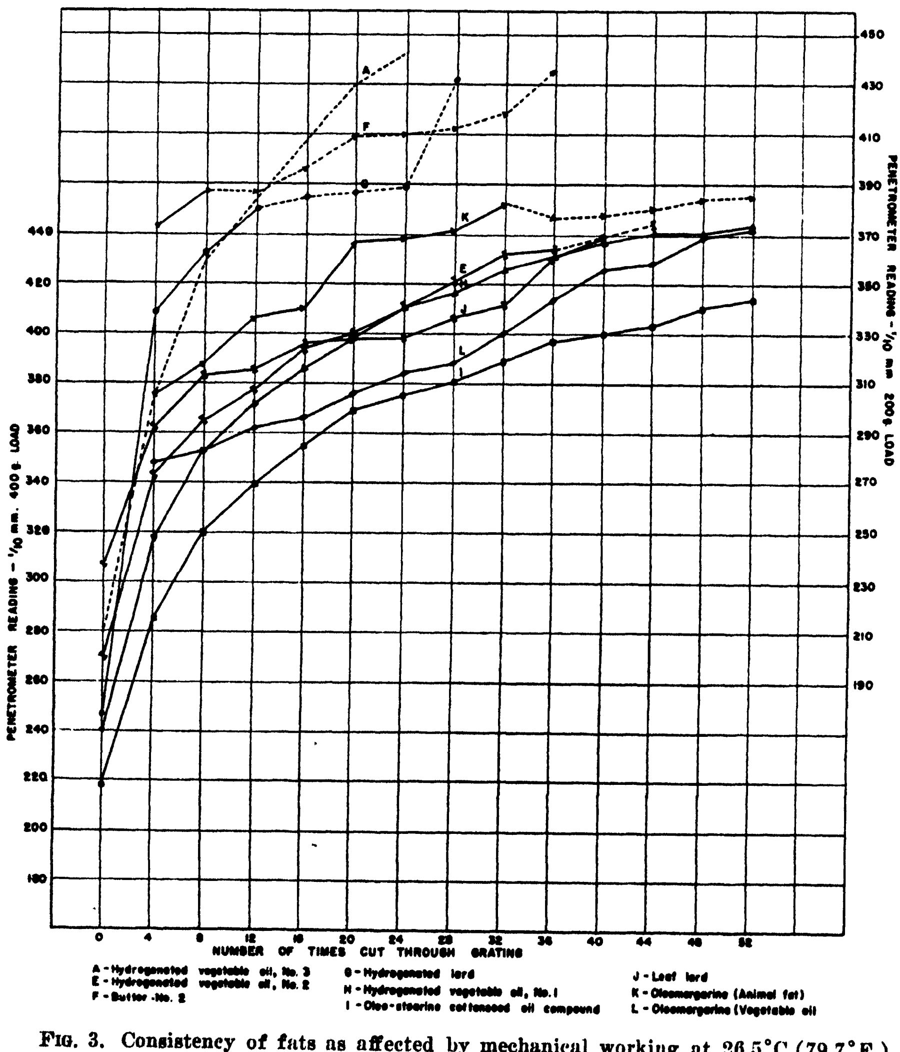

FIG. 3. Consistency of fats as affected by mechanical working at 26.5°C.(79.7°F.). Dotted lines represent readings taken with a 200-gram load.

ity: 18°C., 60 per cent; 22°C., 65 per cent; and 26.5°C., 55 per cent. The hydrogenated lard and the oleomargarines were too hard to be mixed at 18°C. and Sample 1 of butter was too soft to be mixed at 26.5°C.

At least 200 pastries mixed at 22°C. and 100 pastries mixed at 26.5°C. were broken for each fat.

Pastry dough was also mixed by hand at 22°C. and 55 per cent relative humidity in order to prepare a product similar to that made by the homemaker. The formulas used in machine mixing were retained. Fat, flour, and salt were cut together with a pastry blender until the mixture resembled coarse corn meal. This operation required about one minute and 15 seconds. By mixing to the same state of division in each case, differences in the ease of mixing the various fats with flour were eliminated. The water was added and combined with the other ingredients during the next minute. Another minute was required for shaping the dough into a loosely packed ball with the hands. Rolling and cutting were carried out as before. The handmade pastries were baked at 218°C. for 13 to 14 minutes or until lightly browned on the edges.

One-half of the 20 pastries in each baking were broken on the shortometer. The remainder was judged by a panel of selected judges in order to determine whether there was an agreement between the breaking strengths of the pastries and the judges' estimation of tenderness.

An analysis of variance of breaking strength of pastry prepared under the various conditions described above showed that in all cases there was a highly significant difference between the breaking strengths of the pastries made from the different fats. There was a significant difference between the breaking strengths of the 20 pastries made from each batch of dough and also between those of the 10 pastries cut from the two rolls. However, the effects of the different mixings and rollings were minor when compared with the effect of the kind of fat on the breaking strength of the pastries.

Modified Shortbread: In shortbread the fat is creamed before being combined with the other ingredients and forms an emulsion with the water during mixing. The shortbread formulas were as follows:

	100-per cent fats	80-per cent fats
Flour (all-purpose or family)	300 gm.	300 gm.
Fat	115 gm.	144 gm.
Water	60 ml.	47 ml.
Sugar	125 gm.	125 gm.

The mixing of the shortbread was performed in a room having a temperature of 22°C.(71.6°F.) and a relative humidity of 65 per cent.

The fat was creamed for one minute at medium speed in the 10-quart container of a mixer using the flat beater. The sugar was added during the next minute of beating, and creaming of the mixture was continued for five more minutes. The beater was stopped and changed to low speed, and the sides of the container were scraped down. One-third of the flour and all of the water were added and the mixture was beaten for one minute. The remaining flour was added in two equal portions and beating of the mixture was continued for one-half minute after the first addition and

one and one-half minutes after the second addition. The beater was stopped and the sides of the container scraped down before each addition of flour. One-half of the dough was rolled, cut, and placed on the baking sheet in the same manner as for pastry. The shortbread was baked for 10½ minutes at 193°C.(379.4°F.) and cooled for 15 minutes. Each piece was broken on the shortometer. The remaining dough, which had been standing about one hour, was rolled, cut, and baked in the same manner.

The necessity of two bakings for each lot of dough introduced an additional source of variance in the case of shortbreads beyond that present when pastries were the test product. That this factor caused a decrease in the uniformity of the products was definitely indicated by the analysis of variance. However, the effects of all other variables were again minor in comparison with the effect of the kind of fat used. In all cases there was a highly significant difference between the breaking strengths of the pastries made from the different fats.

COMPARISON OF RESULTS

Breaking strengths of test products and their relationship to fundamental properties of the fats are shown (Table 1). The fats are listed in increasing order of breaking strengths of pastries mixed at 22°C. by machine. At that temperature hydrogenated vegetable oil No. 3 was the best shortening agent. Refined steam-rendered lard, the butter oil, and butter No. 1 occupied second place. Hydrogenated vegetable oil No. 2, butter No. 2, and hydrogenated lard ranked third. Hydrogenated vegetable oil No. 1 and oleo-stearine cottonseed oil compound came next, then leaf lard and oleomargarine made from animal fat. Oleomargarine made from vegetable oil was the poorest shortening agent for pastry mixed at 22°C.

The temperature at which pastry dough was mixed affected the shortening value of some fats much more than that of others. The butter fats were most affected. They were relatively poor shortening agents at 18°C. but ranked among the best at 22 and 26.5°C. Hydrogenated vegetable oil No. 3 and refined steam-rendered lard were only slightly affected by changes in temperature, however, and were excellent shortening agents at all three temperatures studied. The oleomargarines produced the least tender pastries at 26.5°C. just as they did at 22°C. The other fats changed in shortening value by variable amounts as the temperature was raised.

No relationship was evident between the breaking strengths of shortbreads and those of pastries mixed at 22°C., or between those of pastries mixed by machine and those mixed by hand at 22°C. The judges' estimation of the shortening value of the fats as determined from the score for tenderness differed from that determined from the breaking strengths of the pastries.

It is apparent that there is no correlation between breaking strengths of either pastries or shortbreads and the physical and chemical constants shown (Table 1). The degree of unsaturation of a fat appeared to be a poor measure of its shortening power. Although the fat with the highest iodine number was an excellent shortening agent at all temperatures used, the three fats next in order of iodine number ranked in the lower half as shortening agents. Butter oil, on the other hand, with the lowest iodine

TABLE 1

Mean Breaking Strength of Machine-Mixed Pastries and Shortbread and Mean Breaking Strength and Mean Score for Tenderness of Hand-Mixed Pastry Made From 11 Commercial Fats, and Some Chemical and Physical Constants of the Fats

Fat	Mean breaking strength					Mean score for tenderness of hand-mixed pastry	Chemical and physical constants			
	Pastries			Shortbread	Pastries					
	Machine mixed at 18°C.	Machine-mixed at 22°C.	Machine-mixed at 26.5°C.	Machine-mixed at 22°C.	Hand-mixed at 22°C.		Iodine number (Hanus)	Free fatty acids as oleic	Melting point	Congealing point
	oz.	oz.	oz.	oz.	oz.			pct.	°C.	°C.
Hydrogenated vegetable oil No. 3	14.1	12.5	13.9	33.9	14.5	2.8	95.7	.036	51.5	36.7
Refined steam-rendered lard	14.0	13.3	10.8	37.4	13.9	3.6	65.0	.369	45.0	26.7
Butter oil from butter No. 2	17.9	13.4	12.8	40.8	13.2	3.6	33.2	.264	34.8	24.3
Butter No. 1	19.7	13.6	......[2]	35.8	16.9	3.6	41.1	.221	35.8	24.6
Hydrogenated vegetable oil No. 2	14.9	14.4	13.4	34.2	18.8	3.2	63.2	.032	46.4	30.2
Butter No. 2	16.9	14.7	14.9	38.4	16.9	4.1	33.2	.264	34.8	24.3
Hydrogenated lard	......[1]	14.8	14.8	34.3	15.5	2.9	58.4	.034	47.3	33.0
Hydrogenated vegetable oil No. 1	17.3	18.0	14.9	40.5	14.9	3.4	71.2	.026	46.6	33.1
Oleo-stearine cottonseed oil compound	16.6	18.1	13.9	38.6	18.5	3.0	80.0	.087	48.8	37.2
Leaf lard	17.1	18.8	15.2	48.2	16.8	3.5	57.0	.257	49.4	32.9
Oleomargarine (animal fat)	......[1]	18.8	19.8	38.7	21.9	2.7	52.8	.323	37.9	26.5
Oleomargarine (vegetable oil)	......[1]	25.2	21.5	42.9	22.0	3.7	66.4	.268	36.8	26.9
Necessary difference for probability of 5%	0.96	0.64	0.95	1.30	2.00	0.68	......	......	......	......
Necessary difference for probability of 1%	1.26	0.84	1.25	1.71	2.63	0.91	......	......	......	......

[1] The fats were too hard for mixing at 18°C. [2] Butter No. 1 was too soft to use at 26 5°C.

number of all the fats, was one of the best shortening agents at the higher temperatures. Congealing points and melting points were also poor criteria of the shortening power of the fats.

There seemed to be no relationship at any of the temperatures used between the breaking strengths of pastries and the consistency of the undisturbed fats. At 22°C., however, there was a highly significant correlation between the breaking strengths of machine-made pastry and the consistency of the worked fat. The coefficients of correlation between the breaking strengths and the worked consistency calculated at 8, 24, and 40 cuttings were —.875, —.837, and —.841, respectively. The correlation was less significant at 18 and 26.5°C., and it is possible that the values for both consistency and breaking strength were less reliable at these temperatures. Only two determinations of each point on the consistency curves were made at 18 and 26.5°C. as against five determinations each at 22°C. In addition the pastries themselves were more difficult to prepare at the two extremes of temperature, being very hard to roll out at 18°C. and so soft that they were sticky and hard to handle at 26.5°C. Nevertheless, coefficients of correlation between breaking strengths of pastries and consistency readings at 8, 24, and 40 cuttings, combining values for all three temperatures, were —.593, —.527, and —.574. These values are highly significant and indicate that fats which become softest during the process of being incorporated into a dough have a tendency to form the most tender pastries.

There was no significant correlation between the breaking strengths of shortbreads and either the consistency or the creaming power of the fats. If any of the physical or chemical properties of the fats determined in this experiment influenced shortening value when the fats were made into shortbreads, the influence was masked by some other factor not studied.

DISCUSSION

The results of this study differ in several respects from those obtained by previous workers. In most studies the hydrogenated fats have been shown to be greatly inferior to the lards in shortening value. Although prime steam-rendered lard ranked first in shortening value in the present experiment, hydrogenated vegetable oil No. 3 was about as good, and all three of the hydrogenated vegetable oils were found to equal or to excel leaf lard in shortening power. Improvement in the quality of hardened fats may be responsible for this change. The butterfats were also found to be very good shortening agents in this study, particularly at the higher temperatures. Butter has usually been considered poor in this respect and Lowe *et al.* (1938) found it to have the lowest shortening value of the fats used. Because of correction of the formula for the increased water and lower fat content of butter, the pastries made from butterfats in this experiment contained a higher proportion of fat than those reported on by Lowe, so that results are not strictly comparable. Finally, there was no correlation between the breaking strengths of pastries and either the congealing points or the iodine numbers of the fats. Of the fundamental properties of the fats, only the consistency of the worked fats, probably a measure of what has been termed "plasticity," was found to correlate with shortening power.

The enclosure of a large amount of liquid in a relatively small amount of solid is characteristic of systems that exhibit thixotropic behavior, [Freundlich (1935)], and solid fats are made up of a loose conglomeration of fine crystalline needles between which a large quantity of liquid is retained by capillarity. It seems probable, therefore, that the value obtained for the worked consistency of a fat is dependent upon the ratio of liquid to solid fat. The close correlation between the worked consistency of the fats and their shortening value at 22°C. and the tendency for a correlation at 18 and 26.5°C. seem to indicate, then, that the shortening power of a fat may depend either upon the amount of liquid glycerides present or upon the ratio of liquid to solid glycerides.

If this view is correct, it is easy to see why the relationship of shortening value to iodine number has been found to be variable. The liquid fat concentration at any given temperature depends on the melting points of the triglycerides. These are governed in turn by the melting points of the constituent fatty acids and are usually determined by the mean molecular weight of the acids and by the degree of unsaturation. In a group of fats of essentially the same fatty acid composition, such as a series of lards, therefore, the liquid glyceride contents should be directly proportional to the iodine numbers at any temperature. Neither the hydrogenated fats nor the butterfats would be expected to fit into this series, however. During hydrogenation there is a certain amount of elaidinization or conversion of liquid unsaturated acids into their more stable, higher melting, geometrical isomers. In addition, hydrogenated fats may also contain some unchanged linoleic acid to raise the iodine number disproportionately to the amount of liquid fat formed. The butterfats, on the other hand, are characterized by important amounts of butyric and other saturated acids of low molecular weight which tend to increase the liquid glyceride content of these fats while maintaining a low iodine number. In previous studies where a correlation of shortening value with iodine number has been reported, the number of hydrogenated fats used has been limited and the values obtained from them have usually been most out of line with the observed correlation. No correlation between shortening value and iodine number has been reported when only hydrogenated fats were studied. All except two of the fats used in this study were either hydrogenated fats or butterfats so that the absence of correlation of iodine number with shortening value is not surprising.

The behavior of hydrogenated vegetable oil No. 3 as a shortening agent was in accord with its known glyceride composition. This fat was an excellent shortening agent at all temperatures and showed very little change in shortening power with changes in temperature. Since it consisted of about 10 per cent of fully hydrogenated cottonseed oil dispersed in the oil, the ratio of liquid to solid glycerides was high. Owing to the high melting point of the solid material, this ratio was subject to very little change in the temperature range used in this experiment. The greatly increased shortening power of the butterfats and particularly of butter oil with increase in temperature can only be explained by their physical structure. The large quantity of low-melting compounds present in these fats probably causes the liquid glyceride content to increase more rapidly with

rise in temperature than is true of most other fats and consequently causes a greater increase in the shortening value.

Enough evidence seems to exist to invalidate the theory that unsaturation plays a deciding factor in determining the shortening power of fats. However, the data now available on the structure and composition of fats used as shortening agents are extremely meager. Fundamental research on these lines is vitally necessary before the property or properties responsible for differences in shortening power can be established.

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EFFECT OF DIFFERENT COOKING METHODS ON THE VITAMIN C CONTENT OF QUICK-FROZEN BROCCOLI [1,2]

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(Received for publication, June 26, 1942)

Little was found in the literature concerning the vitamin C content of fresh, quick-frozen, and cooked broccoli.

McHenry and Graham (1935) report that fresh broccoli contains 68 mg. of ascorbic acid per 100 grams. Roe (1936) reports 68 to 71 mg. per 100 grams. A more recent survey on the vitamin C content of broccoli, throughout one year, as it arrived on the Boston market was made by Feener, Palmer, and Fitzgerald (1937). They report values about twice as high as the above investigators: 113 to 174 mg. per 100 grams.

Wheeler, Tressler, and King (1939) studied the vitamin C content of fresh and stored broccoli. They found 114 to 145 mg. of vitamin C per 100 grams of the fresh vegetable.

Jenkins, Tressler, and Fitzgerald (1938) studied the loss of ascorbic acid from quick-frozen broccoli during storage at four temperatures. They found approximately 90 mg. of ascorbic acid per 100 grams of the frozen broccoli at the beginning of the study. Samples held for six months at —40°C.(—40°F.) showed no loss; samples held for six months at —18°C. (—4°F.) showed only a slight loss; while those samples held for six months at —12°C.(10.4°F.) retained only 65 mg. of ascorbic acid per 100 grams, or 72 per cent; and those samples held for five months at —9°C.(15.8°F.) retained only 12 mg. of ascorbic acid per 100 grams, or 13 per cent.

Fenton and Tressler (1938) found very little destruction of vitamin C from frozen peas during cooking but an appreciable loss to the cooking water, about 36 per cent.

McIntosh, Tressler, and Fenton (1940), working on the effect of different cooking methods on the vitamin C content of quick-frozen Brussels sprouts, cauliflower, lima beans, peas, and spinach, report that in all experiments, except when lima beans were cooked in a standard pressure cooker, the vitamin C destruction was less than 15 per cent.

[1] This paper represents work supported by a grant from the Frosted Foods Sales Corporation, New York City, and utilized Birdseye broccoli (quick-frozen) supplied by the Central Laboratories, General Foods Corporation, Hoboken, New Jersey.

Acknowledgment is made to the National Pressure Company, the Vischer Products Company, and the Carrollton Metal Products Company for utensils used during the investigation.

[2] Approved by the director of the New York State Agricultural Experiment Station as Journal Paper No. 514, June 2, 1942.

The purpose of this study was to determine (1) the vitamin C content of commercially quick-frozen broccoli (a) in the frozen state and (b) defrosted, and (2) the effect on the vitamin C content of (a) different methods of cooking, (b) varying the composition of the cooking utensil, and (c) storing the cooked broccoli in a mechanical refrigerator.

EXPERIMENTAL PROCEDURE

Frozen Broccoli Studied: Broccoli of the Italian Green Sprouting variety was used. It was harvested and frozen September 23, 1939, in Hillsboro, Oregon. The broccoli was part of a regular commercial pack prepared and frozen for the Frosted Foods Sales Corporation. It was cut into four and seven-eighths inch lengths, measuring from the tip or head, and was trimmed to remove the attached leaves and leaf stems. The small inner leaves were not removed but the leaves extending beyond the head were cut back. The broccoli was steam-blanched and was frozen in a Birdseye multiplate freezer. The approximate temperature of the plates of this machine was —33.3° C. (—28° F.). Prior to the receipt of the samples at the laboratory, they were stored in a commercial refrigerated warehouse maintained at —17.8°C.(0°F.). While the vegetable was being studied it was stored in a cold room which was maintained at —21.7 to —23.3°C.(—7 to —10°F.).

Sampling: To determine the variation in the vitamin C content of the various parts of the broccoli, and the variation from stalk to stalk, analyses were made on the buds alone, upper part of the stem, lower part of the same stem, and stems of different sizes and intensities of color.

In order to obtain a fairly representative sample a slice from one-half to three-fourths inch thick was removed from the side of each package of frozen broccoli. Previous work showed that slices from the center and the edges of the package contained about the same amounts of vitamin C. The variation in the vitamin C content between the parts of an individual stalk, as well as between stalks, made it advisable to include representative parts of the buds and stems of at least three stalks of broccoli in each sample. This slice was immediately cut into fine pieces with a sharp knife and a representative sample weighed into the extractant. The remainder of the broccoli from the package was cooked. From the cooked product four stalks were selected at random, cut into fine pieces, and a representative sample weighed into the extractant. Samples of the cooking water were weighed into the chilled extractant.

Chemical Determination: The method of ascorbic acid determination was essentially that of Bessey and King (1933) as modified by Mack and Tressler (1937).

The extractant used was 1 N sulphuric acid containing two per cent metaphosphoric acid. It was found that the 1 N sulphuric acid containing two per cent metaphosphoric acid gave more complete extraction of the vitamin C than either eight per cent acetic acid containing two per cent metaphosphoric acid or eight per cent trichloroacetic acid containing two per cent metaphosphoric acid, or five per cent metaphosphoric acid. Because complete extraction of the vitamin C could not be accomplished with larger samples, a five-gram sample was used. Duplicate samples

were weighed into 20 ml. of the extractant. Acid-washed sand was used to facilitate grinding the samples. Five extractions were made for each sample, using five 20-ml. portions of the extractant. The vegetable extract was made up to 100 milliliters. The samples of the hot cooked vegetable and of the hot cooking water were added to chilled extract so that they were brought to room temperature immediately. A pipette was used to transfer 10 grams of cooking water into 25 ml. of the extractant in a 50-ml. volumetric flask. This was made up to volume with the extractant.

Aliquots of these extracts were titrated against a solution of 2.6 dichlorophenolindophenol dye. The dye was standardized daily against pure ascorbic acid. Blanks were run. Since the body is able to utilize dehydroascorbic acid as well as the reduced form of ascorbic acid, aliquots of the vegetable extracts and of the cooking water were treated with hydrogen sulphide, using a modification of the method of Tillmans, Hirsch, and Jackisch (1932). Blanks were run with this treatment. In every titration a large enough aliquot was used to give a titration value of over one milliliter of the dye.

Since no dehydroascorbic acid was found in the quick-frozen broccoli either before or after cooking, it was decided not to make routine analyses for it. Instead tests were made each month during the study. At no time was any dehydroascorbic acid found.

Cooking Utensils: The following types of cooking utensils were used in this study:

1. Saucepans, two-quart capacity, approximately five and one-half inches in diameter and five inches deep.
   - A. Enamel, uncovered and pyrex cover.
   - B. Stainless steel, uncovered.
   - C. Aluminum, uncovered.
   - D. Pyrex, uncovered.
2. Steamers, aluminum, four-quart capacity.

   Steamer A: The upper compartment had perforations in the floor so that both the frost from the package and the condensed steam dripped into the lower compartment.

   Steamer B: The upper compartment was constructed so that the steam entered above the vegetable and both the frost from the package and the condensed steam remained with the vegetable.
3. Pressure saucepans, two-quart capacity.
   - A. Presto pressure saucepan, aluminum alloy. This saucepan was especially equipped with a standard pressure gauge in addition to the pressure indicator on the weight. A maximum thermometer was used to record temperature.
   - B. Flex-seal pressure saucepans.
     1. Aluminum.
     2. Stainless steel.

     To record the temperature the rubber safety disc in the cover of each of these was replaced by a thermometer inserted in a rubber stopper.
4. Standard pressure cooker, 10-quart capacity.
   This was equipped with a pressure gauge and a thermometer.

Preparation for Cooking: In most cases the broccoli was cooked without any defrosting. When the broccoli was to be steamed and in some of the studies where it was cooked in the pressure saucepans, it was defrosted,

only enough to allow separation of the stalks, by allowing it to stand at room temperature, 25.6°C.(78°F.), for one hour and a quarter.

Preliminary determinations were made to find the effect of allowing the broccoli to defrost in the following ways:

1. Standing at room temperature (78°F.) for one and one-fourth hours.
2. Standing in a mechanical refrigerator for 16 hours at 4.4°C.(40°F.).

In each case the seal of the package had been broken to remove the sample of solidly frozen broccoli, but the box was again closed and the outer wrapper held in place by rubber bands.

Cooking Methods: In each of the cooking studies the pan and a gas burner were placed on a balance so that the weight of the pan and its contents could be obtained during each stage of cooking. A manometer was connected between the gas supply and the burner so that it was possible to duplicate the amount of evaporation in the various cookings (10). Amounts of broccoli for four servings (about 300 grams) were cooked each time. The frost was left with the vegetable and was included with the weight of the frozen vegetable. The amount of frost varied from package to package; it measured from zero to three-eighths of an inch in depth in the bottom of the packages. In the studies where the broccoli was slightly defrosted before cooking, the frost was still solid and it was added to the cooking utensil with the vegetable. In the boiling studies the solidly frozen broccoli was dropped into a weighed amount of rapidly boiling water. The time was counted from the time when the water came back to the boil which required approximately five and one-half minutes. The broccoli required five and one-half minutes' boiling to reach the done stage. The cooked broccoli was drained 30 seconds in an enamel colander and the weights of the cooked vegetable and the cooking water were determined.

To determine whether covering the pan had any effect on the vitamin C present, the broccoli was boiled in 500 grams of water in both a covered and an uncovered enamel pan.

The effect of varying amounts of water, used in cooking, was determined by boiling broccoli in an enamel pan, using 100, 500, and 1,000 grams of water. In order to cover the solid block of frozen broccoli 500 grams of water were necessary. When 100 grams of water were used it was necessary to cover the pan to insure uniform cooking and to prevent the vegetable from cooking dry and scorching.

To determine the effect of cooking for different lengths of time the broccoli was boiled in an uncovered enamel pan for two, five and one-half, and 11 minutes. These times gave products which were undercooked, done, and overcooked.

The effect of the composition of the cooking utensil was studied by boiling broccoli in 500 grams of water in uncovered saucepans of enamel, pyrex, aluminum, and stainless steel.

For steaming, water was placed in the lower part of each steamer, the inset pan and cover were placed in position, and the water was brought to the boil so that both compartments were filled with steam before the vegetable was added. Where there was liquid remaining in the bottom of the inset pan it was also analyzed. Sixteen minutes were required to cook

the slightly defrosted broccoli in steamer A, in which steam entered from the bottom, and 17 minutes in steamer B.

Solidly frozen broccoli was cooked in aluminum and stainless-steel Flex-seal pressure saucepans. Broccoli which had been slightly defrosted was cooked in the aluminum Flex-seal and the Presto pressure saucepans. The fact that the Presto pressure saucepan had thicker walls and a thicker cover than the Flex-seal pressure saucepans probably had little influence in this study, because both types of pressure saucepans including the covers were thoroughly heated and the water was boiling rapidly when the broccoli was added. Furthermore, at the end of the cooking period the pans were removed from the stove, set in cold water, and the covers removed immediately. No more than 45 seconds elapsed between the time the pan was removed from the heat and the broccoli was taken out of the pan. Consequently the actual time of cooking in the two types of pans was approximately the same.

In all cases 30 grams of water were brought to the boil and the pan thoroughly heated before the vegetable was added and the cover brought into place. The weight was placed on the vent each time when the temperature inside the pan reached 98.9°C.(210°F.). The boiling point of water in this laboratory was found to be 210°F. This temperature was determined in the Presto pressure saucepan by a maximum thermometer and in the Flex-seal pressure saucepans by a thermometer inserted in the covers. It was thus possible to check the pressure of 15 pounds against a temperature of 119.4°C.(247°F.). Thirty grams of water were found to be the smallest amount which could safely be used and scorching prevented. The directions which came with the pans called for one-fourth cup (59 gm.) for fresh broccoli.

When solidly frozen broccoli was used, five and three-fourths minutes were required to bring the saucepans to 15 pounds pressure, and two and three-fourths minutes at 15 pounds pressure were required to give a product which was done. With the slightly defrosted broccoli five and one-fourth minutes were required to bring the pressure to 15 pounds and only three-fourths minute at 15 pounds pressure was required to give a done product. As soon as the cooking period was completed the pressure saucepans were set in cold water, the weight removed from the vent, and the cover removed. In no case did this procedure require more than three-fourths of a minute.

Although it is well known that the sulfur-containing vegetables such as broccoli cannot be satisfactorily cooked in a pressure cooker, it was thought to be of theoretical interest to determine the vitamin C losses from quick-frozen broccoli by this method.

The standard pressure cooker was equipped with a pressure gauge and thermometer and was controlled in the same manner as the pressure saucepans, with the exception that, owing to its size, it was not immersed in cold water to bring the pressure back to zero. Instead the valve was opened immediately to allow the steam to escape. From one to one and one-half minutes were required for the pressure to reach zero after the valve was opened. The pressure cooker required seven minutes to reach 15 pounds

pressure and three minutes at pressure to insure a done product. Only solidly frozen broccoli was cooked by this method.

The inset pan with perforations in the bottom was used. It allowed the steam to come up through the block of broccoli and thaw it more quickly.

Stored, Cooked Broccoli: Since several vegetables have been reported to lose vitamin C on standing, even in a covered dish in a refrigerator, a study was made of the effect of this treatment on cooked broccoli. The broccoli was boiled, a sample analyzed immediately, and the remainder of the cooked vegetable was placed in a covered pyrex casserole in a mechanical refrigerator held at 40°F. Samples were analyzed at intervals of 24 and 48 hours.

Stored, Frozen Broccoli: Incidental to the other studies the effect of storing quick-frozen broccoli for five months at —21.7 to —23.3°C.(—7 to —10°F.) was observed.

TABLE 1

Some Variations in Vitamin C Content of Quick-Frozen Broccoli

Description of sample	Ascorbic acid
	mg./100 gm.
Yellow green bud	49
Dark green bud	67
Small dark green stem	122
Large light green stem	89
Medium size, medium green stem	100

DISCUSSION OF RESULTS [3]

The buds of the frozen broccoli contained less vitamin C per gram than did the stalks. The average value of 14 samples was 60 mg. per 100 grams of buds, 107 mg. per 100 grams of stalks. Samples of buds and stalks together averaged 88 mg. per 100 grams.

It was found, however, that the buds of unblanched broccoli were richer in vitamin C than the stalks. This indicates that there was relatively greater retention of the vitamin C by the stalk during the washing and blanching steps preliminary to the freezing operation. This is probably explained by the fact that the buds have a larger surface area per gram than do the stalks.

Samples taken from the upper three-fourths inch of the stem of frozen broccoli contained as much as 40 mg. more vitamin C per 100 grams of broccoli than samples taken from the lower three-fourths inch of the same stem. The stems and buds varied in vitamin C content from one to another; some values found are shown (Table 1).

[3] In interpreting all percentages it should be remembered that the frost in the packages contained less vitamin C per given weight than did the vegetable. The vitamin C content of each cooking lot (about 300 grams) of vegetable was obtained by multiplying the milligrams of vitamin C per gram of vegetable by the weight of the vegetable plus the frost. Consequently the vitamin C content of the uncooked lot of broccoli was higher when much frost was present. In this case the percentage retention and solution were lower than when the amount of frost was low.

The frost in 10 packages of broccoli had a range of 18 to 30 mg. per 100 grams and an average of 26 mg. per 100 grams. Since this frost was left with the vegetable it became a part of the cooking water. As stated before, the variation in the vitamin C content from one part of the stem to another, and from bud to stalk, made sampling difficult. Sampling was further complicated by the varying amounts of frost present and the fact that the concentration of vitamin C was greater in the broccoli than in the frost.

Defrosting: When a solidly frozen block of broccoli was cooked by steaming or in the pressure saucepans, the outer part was overcooked while the center part was almost raw. By allowing a slight amount of defrosting to take place—only enough to allow the stalks to be separated and not to actually melt the frost—a uniformly cooked product was obtained. It was found that this amount of defrosting could be accomplished, and at the same time no vitamin C would be destroyed, by leaving a package of solidly frozen broccoli at room temperature, 78°F., for an hour and a quarter or in a mechanical refrigerator for four hours at 40°F. Allowing the broccoli to stand overnight, 16 hours, in a mechanical refrigerator at 40°F. gave a more completely defrosted product but resulted in a six-per cent loss of vitamin C. The results of defrosting are recorded (Table 2).

TABLE 2

Effect of Defrosting on Vitamin C Content of Quick-Frozen Broccoli[1]

Time	Temperature	Number of packages	Ascorbic acid		
			Frozen broccoli	Defrosted broccoli	Loss
hr.	*°F.*		*mg./100 gm.*	*mg./100 gm.*	*pct.*
1¼	78 [2]	6	97	97	0
4	40 [3]	8	85	86	0
16	40	9	95	89	6

[1] The seal of the package was broken to remove a solidly frozen sample. [2] Room temperature. [3] Mechanical refrigerator

Covered versus Uncovered Pans: The broccoli boiled gently in a tightly covered enamel pan retained its green color as well as when it was boiled in an uncovered pan. It is a well-known fact that the green colors are "set" during the blanching operation prior to freezing. No unpleasant flavor or odor developed. In each case the broccoli was boiled until just done, five and one-half minutes. The destruction of vitamin C when the broccoli was boiled in a covered pan was 10 per cent (Table 3). In the uncovered pan it was 11 per cent. The amount of vitamin C lost to solution, 30 to 32 per cent, was the same in the covered and uncovered pans.

Effect of Varying Amount of Water Used in Cooking: By using a loosely covered pan it was possible to use a very small amount of water in cooking. The resulting product retained its green color and pleasing odor and flavor when it was cooked only until done. The vitamin C which went into solution was concentrated in a small volume of water and could easily be used. When 100 grams of water were used, 82 per cent of the vitamin C remained in the vegetable and only 10 per cent was in the water and eight per cent was lost. When 500 grams of water were used, enough to

just cover the solidly frozen block of vegetable, only 57 per cent of the vitamin C remained in the broccoli, 32 per cent went into solution, and 11 per cent was lost. The larger volume of water, besides being a less concentrated source of the vitamin C than the smaller volume, was more difficult to use. About 22 per cent more vitamin C dissolved into the cooking water when 500 grams were used than when 100 grams of water were used.

TABLE 3

Effect on Vitamin C Content of Quick-Frozen Broccoli of Boiling in Covered and Uncovered Enamel Pans[1]

Pan	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water	Retention	Solution	Loss
		mg./ 100 gm.	*mg./ 100 gm.*	*mg / 100 gm.*	*pct*	*pct.*	*pct.*
Covered	8	96	61	20	60	30	10
Uncovered	8	89	55	21	57	32	11

[1] Five hundred grams of water were used. Five and one-half minutes were required to bring the water back to the boil. The broccoli was boiled five and one-half minutes.

When 500 grams of water were used there were 21 mg. of vitamin C per 100 grams of cooking water, as contrasted with 65 mg. of vitamin C per 100 grams of cooking water when 100 grams of water were used at the start of the cooking period. One thousand grams of water gave only slightly greater solution than 500 grams of water. In this case 53 per cent remained in the vegetable and 37 per cent went into solution. The three different volumes of water resulted in approximately the same degree of loss, eight to 11 per cent (Table 4).

TABLE 4

Effect on Vitamin C Content of Quick-Frozen Broccoli of Boiling in Different Amounts of Water[1]

Amount of water used	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water	Retention	Solution	Loss
gm.		*mg./100 gm*	*mg./100 gm.*	*mg./100 gm.*	*pct*	*pct.*	*pct*
100	6	86	76	65[2]	82	10	8
500	8	89	55	21	57	32	11
1,000	6	86	48	11	53	37	10

[1] An enamel pan was used. When 100 grams of water were used the pan was covered; other times it was left uncovered. Five and one-half minutes were required to bring the water back to the boil. The broccoli was boiled five and one-half minutes. [2] Less than 100 grams of water remained after cooking.

These findings are in agreement with McIntosh, Tressler, and Fenton (1942) who, working with five frozen vegetables, found that as the volume of cooking water was increased there was progressively more ascorbic acid in the cooking water and less in the vegetable itself. Their work shows that from one quarter to one half of the ascorbic acid content of vegetables may be leached from them when they are boiled in increasing amounts of water.

Effect of Cooking Time on Vitamin C: The broccoli which was boiled two minutes was undercooked and still very firm and green. Broccoli

boiled five and one-half minutes was just done, while that boiled for 11 minutes had a mushy texture, brown color, and an undesirable odor and flavor. The per cent retention of vitamin C was approximately the same for done and overcooked broccoli, 57 and 55 per cent respectively, as shown (Table 5). The per cent solution was also about the same, 32 to 33 per cent. The undercooked vegetable retained 64 per cent of its vitamin C.

TABLE 5

Effect on Vitamin C Content of Quick-Frozen Broccoli of Boiling for Different Lengths of Time[1]

Length of boiling period	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water	Retention	Solution	Loss
min.		*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*pct.*	*pct.*	*pct.*
2	6	89	59	15	64	25	11
5½	8	89	55	21	57	32	11
11	6	91	55	24	55	33	12

[1] Five hundred grams of water and an enamel pan were used. Approximately five and one half minutes were required to bring the water back to the boil.

No increased loss of vitamin C resulted with the longer boiling periods. During the preboiling period and during the first two minutes of boiling, 25 per cent of the vitamin C went into solution; while during the preboiling and five and one-half minutes boiling, 32 per cent went into solution. Similarly, Fenton, Tressler, and King (1937) found the rate of vitamin C destruction and leaching into the cooking water of boiled Swiss chard and peas to be most rapid during the first two minutes of boiling.

TABLE 6

Effect on Vitamin C Content of Quick-Frozen Broccoli of Boiling in Pans of Different Composition[1]

Composition of pan	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water	Retention	Solution	Loss
		mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*pct*	*pct*	*pct.*
Enamel	8	89	55	21	57	32	11
Aluminum	6	85	48	20	55	32	13
Pyrex	8	87	53	20	56	30	14
Stainless steel	6	89	51	20	54	31	15

[1] Uncovered pans and 500 grams of water were used. From five to six minutes were required to bring the water back to boil. The broccoli was boiled five and one-half minutes.

Effect of Pans of Different Composition: Pans of stainless steel, pyrex, aluminum, and enamel gave approximately the same retention of vitamin C in the vegetable, 54, 56, 55, and 57 per cent, respectively, and the same per cent solution (Table 6). There is very little difference in the extent of loss of vitamin C from quick-frozen broccoli owing to cooking in these pans. While the enamel pan consistently gave slightly less loss, eight to 12 per cent, than the other pans, 13 to 15 per cent, this difference is not considered significant as it is within experimental error. McIntosh, Tressler, and Fenton (1942) determined the effect of boiling Brussels sprouts, cauli-

flower, lima beans, peas, and spinach in pans of aluminum, enamel, pyrex, and stainless steel. They obtained similar results, finding that the composition of the utensil had very little effect on the ascorbic acid content of boiled quick-frozen vegetables.

However, Floyd and Fraps (1940) boiled turnip greens in uncovered stainless-steel, pyrex, enamel, and aluminum pans and found losses of 18.3, 17.1, 15.5, and 26.1 per cent of the vitamin C, respectively, thus indicating greater loss in aluminum than in other utensils.

Steaming: Broccoli steamed in steamer A, with perforations in the floor of inset pan, was uniformly cooked and retained its green color. Broccoli cooked in steamer B, with no perforations in the floor of the inset pan, was less uniformly cooked. Broccoli cooked in both of these steamers retained about 80 per cent of its vitamin C, as shown (Table 7).

With steamer B an average of 50 grams of liquid remained with the vegetable after it was cooked. This liquid was composed of condensed

TABLE 7

Vitamin C Content of Quick-Frozen Broccoli Cooked in Two Types of Steamer[1]

Type of steamer	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water	Retention	Solution	Loss
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*	*pct.*	*pct.*	*pct.*
Steamer A, perforated floor	6	88	80	....[2]	79	....[2]	21[3]
Steamer B, floor not perforated	6	84	72	46[4]	80	11	9

[1] Steamer A required 16 minutes, steamer B required 17 minutes of cooking. [2] The melted frost and condensed steam dripped into the lower compartment. There was no liquid present with the vegetable after cooking. [3] This figure includes loss to solution as well as destruction. [4] An average of 50 grams of liquid was present after cooking. This was a combination of melted frost and condensed steam.

steam and the frost which was with the vegetable and contained 11 per cent of the vitamin C, or 46 mg. per 100 grams of vegetable water.

Pressure Saucepans: As stated previously, when solidly frozen blocks of broccoli were cooked in the pressure saucepans it was impossible to obtain a uniformly done product. It took some time for the steam to penetrate to the center of the block and therefore the outside portions were overcooked and brown while the center was still raw. When the broccoli was allowed to defrost sufficiently for separation of the stalks before cooking, a more uniform product with better color, texture, flavor, and odor was obtained. When the solidly frozen broccoli was put on to cook the vitamin C retention was 72 per cent; when the broccoli had been slightly defrosted the retention was 76 to 80 per cent. The solidly frozen broccoli required two and three-fourths minutes and the slightly defrosted broccoli only three-fourths minute at 15 pounds pressure. The loss of vitamin C in the former case was 17 to 18 per cent, in the latter case 11 to 12 per cent (Tables 8 and 9).

Overcooking in the pressure saucepans (247°F.) for even one-half minute gave a brown product with disagreeable flavor and odor. Overcooking at the boiling temperature (210°F.) did not produce such disastrous results.

When all precautions were taken to insure a short cooking period, the pressure saucepans gave an acceptable product with this sulfur-containing vegetable. These precautions included (1) having the broccoli defrosted sufficiently to allow separation of the stalks before they were put on to cook, (2) having the entire pan hot and the water boiling before the broccoli was added, and (3) stopping the cooking as soon as the vegetable was done by setting the pan in cold water and removing the cover immediately, all within three-fourths minute.

TABLE 8

Vitamin C Content of Solidly Frozen, Quick Frozen Broccoli Cooked in Aluminum and Stainless-Steel Pressure Saucepans[1]

Composition of pan	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water[2]	Retention	Solution	Loss
		mg / 100 gm.	*mg / 100 gm.*	*mg / 100 gm.*	*pct*	*pct.*	*pct.*
Aluminum	7	87	70	49	72	11	17
Stainless steel	7	89	72	50	72	10	18

[1] The broccoli was cooked in 30 grams of water in Flex seal pressure saucepans. Five and three-fourths minutes were required to bring the pressure to 15 pounds; two and three-fourths minutes were required at pressure. The product was not acceptable [2] About 50 grams of water remained after cooking. This included the 30 grams that were added and the frost and seepage.

The Flex-seal and the Presto pressure saucepans gave similar results. Approximately the same per cent solution occurred in all studies with the pressure saucepans. The small amount of water, approximately 50 grams, left after cooking was a rich source of vitamin C, containing from 45 to 65 mg. of vitamin C per 100 grams. It contained a total of 25 to 30 mg. of vitamin C. These results differ slightly from those of McIntosh, Tressler,

TABLE 9

Vitamin C Content of Slightly Defrosted, Quick-Frozen Broccoli Cooked in Two "Makes" of Pressure Saucepans[1]

"Make" of pan	Number of packages sampled	Ascorbic acid					
		Frozen broccoli	Cooked broccoli	Cooking water[2]	Retention	Solution	Loss
		mg./ 100 gm.	*mg / 100 gm.*	*mg / 100 gm*	*pct*	*pct.*	*pct.*
Aluminum Flex-seal	9	95	81	53	76	12	12
Presto saucepan	7	94	83	55	80	9	11

[1] The broccoli was cooked in 30 grams of water Five and one fourth minutes were required to bring the pressure to 15 pounds; three-fourths of a minute was required at pressure. [2] About 50 grams of water remained after cooking. This included 30 grams that were added and the frost and seepage.

and Fenton (1940) who found that the vitamin C retention in Brussels sprouts, cauliflower, lima beans, peas, and spinach was highest when they were cooked in the pressure saucepans.

Pressure Cooker: About 58 per cent of the vitamin C was retained in the broccoli cooked in the pressure cooker. Since the inset pan had perforations in the bottom, the frost and condensed steam were not saved. The broccoli cooked by this method was not acceptable; it had a brown color, an undesirable flavor and odor, and a mushy texture. This was no

doubt due not only to the high temperature reached but also to the length of time required to bring the pressure to 15 pounds and to reduce it to zero in this large vessel.

These studies, in general, agree with McIntosh, Tressler, and Fenton (1940) who worked on the effect of the different cooking methods on the vitamin C content of quick-frozen Brussels sprouts, cauliflower, lima beans, peas, and spinach and reported that in all experiments, except when lima beans were cooked in the pressure cooker, the vitamin C destruction was less than 15 per cent. These studies also agree with the work of Fenton and Tressler (1938) who boiled quick-frozen peas. The cooked frosted peas, taken directly from storage at —40°C., retained 59 per cent of vitamin C and 36 per cent was dissolved in cooking water, making a total of 95 per cent retention.

This study shows that the vitamin C lost during the cooking of broccoli is less than 15 per cent except when the solidly frozen broccoli is cooked in the pressure cooker and the pressure saucepans.

TABLE 10

Losses of Vitamin C From Boiled, Quick Frozen Broccoli Upon Standing[1]

Number of packages sampled	Ascorbic acid				
	Hours of storage			Loss	
	0	24	48	After 24 hours	After 48 hours
	mg /100 gm	*mg /100 gm*	*mg /100 gm*	*pct*	*pct*
6	77	62	51	19	34

[1] In a loosely covered dish in a mechanical refrigerator at 4.4°C (40°F)

Stored, Cooked Broccoli: Cooked broccoli lost vitamin C on standing in the refrigerator at 40°F. In 24 hours 19 per cent and at the end of 48 hours 34 per cent of the vitamin C had been lost, as shown (Table 10).

Stored, Frozen Broccoli: There was no loss of vitamin C from the stored, quick-frozen broccoli during this study. It was held at —17.8 to —23.3°C.(0 to —10°F.) for five months from February to August. Jenkins, Tressler, and Fitzgerald (1938) studied the losses of vitamin C from frozen broccoli during storage and reported that there was a small loss during the first two months' storage at —18°C.(0°F.) and none thereafter. Since the broccoli used in this study had been stored for more than two months prior to its arrival in the laboratory it agrees with this study.

SUMMARY

The vitamin C content of different stalks of the quick-frozen broccoli varied from 74 to 100 mg. per 100 grams and averaged 88 mg. per 100 grams.

The upper three-fourths inch of the stalks contained as much as 40 mg. per 100 grams more vitamin C than the lower three-fourths inch.

The stalks of the quick-frozen broccoli contained more vitamin C than the buds. Buds averaged 60 mg. per 100 grams and the stems averaged 107 mg. per 100 grams. The reverse was true, however, in fresh raw broccoli from another lot where the buds were the richer source of vitamin C.

The vitamin C content of the frost in the packages of quick-frozen broccoli varied from 18 to 30 mg. per 100 grams. The amount of frost in each package was small (10 to 30 gm.) when compared with the weight of the vegetable.

In steaming and cooking in the pressure saucepans it was necessary to defrost the broccoli sufficiently to allow the stalks to be separated in order to secure a uniformly done product from the standpoints of color, odor, flavor, and texture. This slight defrosting was accomplished, with no destruction of vitamin C, by allowing the vegetable to stand at room temperature, 78°F., for one and one-fourth hours or in a mechanical refrigerator at 40°F. for four hours.

When broccoli was defrosted in the refrigerator at 40°F. for 16 hours, six per cent of the vitamin C was lost.

When solidly frozen broccoli was boiled in 500 grams of water in an enamel pan it retained about 60 per cent of its vitamin C. Covering or uncovering the pan made no appreciable difference in the loss or solution of the vitamin.

When solidly frozen broccoli was boiled in a covered enamel pan in only sufficient water to keep it from burning, 100 grams, it retained 82 per cent of its vitamin C. When boiled in 500 grams of water in an uncovered enamel pan, it retained 57 per cent of its vitamin C. When boiled in 1,000 grams of water in an uncovered enamel pan it retained 53 per cent of its vitamin C. The extent of loss was approximately the same with 100, 500, or 1,000 grams of water. The variation was in the amount of vitamin C that went into solution

Overcooking for two minutes at the high temperature attained in the pressure saucepans caused an increased loss of five to eight per cent. This loss is probably due to oxidation of the vitamin C at 247°F. It is impossible to drive all the oxygen out of the cooking utensil and vegetable tissue before building up pressure. Slight overcooking in boiling broccoli did not cause increased vitamin C loss.

When quick-frozen broccoli was boiled in pans of different composition there was little difference in the vitamin C retention. The enamel pan consistently gave less loss, eight to 12 per cent, than did the pans of stainless steel, aluminum, and pyrex, 13 to 15 per cent. This difference is within experimental error. The composition of the pan made no difference in the amount of the vitamin C dissolved.

Slightly defrosted broccoli, when cooked in either steamer, retained about 80 per cent of its vitamin C. When steamer B was used the frost and condensed steam contained about 11 per cent of the vitamin C, or 46 mg. per 100 grams.

Solidly frozen broccoli cooked in the pressure saucepans retained 72 per cent of its vitamin C, 17 to 18 per cent was lost. The product was not uniformly cooked.

Slightly defrosted broccoli cooked in the pressure saucepan retained 76 to 80 per cent of its vitamin C, 11 to 12 per cent was lost. The product was acceptable. The stainless-steel and aluminum Flex-seal and the Presto pressure saucepans gave comparable results.

Broccoli cooked in the standard pressure cooker retained 58 per cent of its vitamin C but was not an acceptable product.

Cooked broccoli lost 19 per cent of its vitamin C on standing in the refrigerator at 40°F. for 24 hours and 34 per cent on standing for 48 hours.

The quick-frozen broccoli stored at 0 to —10°F. did not lose vitamin C during five months' storage.

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PEANUT BUTTER AS A SOURCE OF THIAMIN, CALCIUM, PHOSPHORUS, AND IRON [1]

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Large quantities of peanut butter are produced and consumed in the United States. No figures are available on the per-capita consumption, but a report of the Department of Commerce (1940) shows that 156,896,063 pounds of peanut butter, valued at $16,668,066, were produced in the United States in 1939.

At the time the study here reported was begun, most vitamin tables gave values for unroasted peanuts only, and little or no data were available on the calcium, phosphorus, and iron contents of peanut butter.

Reports in the literature indicate a wide variation in the thiamin content of peanuts and peanut butter. Borsook (1941), who does not indicate the source of his original data, reports the thiamin content of peanut butter as 125 international units for one and one-half ounces (approximately 870 micrograms per 100 grams). Sherman (1941) reports both roasted peanuts and peanut butter to contain 500 to 600 micrograms per 100 grams. Booher and Hartzler (1939) give no data on peanut butter but found whole-roasted Spanish peanuts to contain 234 micrograms per 100 grams. Munsell (1940) reports 180 and 270 micrograms of thiamin per 100 grams of roasted peanuts.

Using the chemical method of Melnick and Field, Higgins *et al.* of the Georgia Experiment Station (1941) determined the thiamin content of 29 samples of raw peanuts, two samples of roasted peanuts, and 13 of peanut butters. They report the thiamin content of peanut butters on the moisture-free basis to vary from .4 to 1.3 micrograms per gram with an average of .7 microgram per gram. Since peanut butter contains one to two per cent of moisture, the thiamin content of the peanut butter as used would, according to their report, be a little less than 70 micrograms per 100 grams.

The Georgia Experiment Station also reports analyses of the mineral contents of peanuts but not of peanut butters. It is not clearly indicated, but presumably the chemical analyses were made on raw peanuts including the brown skin. The average calcium content of 29 samples was .047 per cent, and the average phosphorus content of 28 samples was .49 per cent. No data on the iron content of peanuts are given. Sherman (1941), McCance *et al.* (1936), and Bridges (1935) all report mineral values for peanuts, but it is not known whether the analyses were made on raw or roasted peanuts with or without skins. The calcium contents of peanuts are stated to be .066, .061, and .071 per cent by the respective authors.

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[1] Published with the approval of the director as Technical Paper No. 102 of the Hawaii Agricultural Experiment Station.

[2] Resigned.

Their values for phosphorus are .392, .365, and .399 per cent and for iron .0019, .00204, and .00231 per cent. Bridges (1935) gives the only figures that could be found for the mineral content of peanut butter—.072 per cent calcium, .396 per cent phosphorus, and .0018 per cent iron.

This paper presents data for the thiamin, calcium, phosphorus, and iron contents of four commercial peanut butters.

EXPERIMENTAL PROCEDURE

Requests were addressed to manufacturers for information as to the varieties of peanuts and the methods of preparation used. A summary of their reports follows:

No. 1. This peanut butter is made from equal proportions of Spanish [3] and Virginia [3] peanuts. Shelled raw peanuts are roasted in gas ovens for one hour at a temperature of 176.7 to 190.6°C.(350 to 375°F.) and cooled. Hulls, skins, and hearts [4] of the peanuts are removed by mechanical means, and the cleaned peanuts are conveyed to the grinding machines, ground into peanut butter, and placed at once in containers for the trade. One-half of one per cent salt is added during the grinding.

No. 2. This peanut butter is made from a blend of equal parts of No. 1 Spanish and No. 1 Virginia peanuts. The nuts are roasted at a temperature of approximately 121.1°C.(250°F.) for 50 to 60 minutes depending upon the amount of moisture in the peanuts. After the peanuts are roasted and cooled they are put through the cleaner and blancher to remove the hulls and peanut eyes.[4] They are then sent over a conveyor and hand picked to remove shriveled and discolored peanuts before grinding. The company did not report the addition of salt or oil, but the label on the jar used stated that the peanut butter contained salt and one-half of one per cent added hydrogenated peanut oil.

No. 3. This peanut butter is made from 75 per cent Virginia peanuts and 25 per cent Spanish peanuts. The two different varieties of shelled raw nuts are roasted separately using a temperature of 287.5 to 343.3°C. (550 to 650°F.). The time of roasting varies from 45 to 60 minutes depending mainly on the initial temperature of the peanuts. After roasting, the peanuts are cooled and blanched, and the skins and hearts removed. The peanuts are mixed in proper proportions and ground with salt (about two per cent) and a small percentage of hydrogenated peanut oil. The product is placed in vacuum-capped containers.

No. 4. This peanut butter is made of two-thirds Spanish and one-third Virginia peanuts. The shelled peanuts are roasted in 500-pound batches in gas-heated revolving roasters at a temperature of 148.9 to 204.4°C.(300 to 400°F.) for about 45 minutes. The red skins are removed. (The company did not state whether the hearts are removed and discarded.) Foreign material is removed mechanically and by hand before the roasted peanuts

[3] It is assumed that when the manufacturers report the use of Spanish and Virginia peanuts, these proper names refer to types or varieties of peanuts and not to their origin, as no peanuts are imported from Spain and the Virginia varieties are grown in a number of states.

[4] The terms "hearts," "eyes," and "germs" are used by the trade to mean those portions of the peanut which botanically are the epicotyl and hypocotyl and do not include the cotyledons.

are ground. A coarse grind leaves pieces of unground peanuts (about 40 per cent by weight) mixed with a larger proportion of smoothly ground nuts.

Thiamin, moisture, total ash, and the ash constituents were determined as previously described for macadamia nuts by Miller and Louis (1941).

RESULTS AND DISCUSSION

The results of the feeding experiments and the thiamin values of peanut butter are summarized (Table 1). The assays of Series 1 show a maximum of 450 micrograms and a minimum of 350 micrograms, or an average of 381 micrograms per 100 grams of peanut butter. The difference between Samples 2, 3, and 4 is less than five per cent. The greatest difference, that between Samples 1 and 2, is a little more than 20 per cent. Considering that these were commercial products of different origin, the variation in the thiamin values is not great. A different sample of peanut butter No. 2 was tested a year and a half after the first series and the difference found to be less than 10 per cent. This is probably within the error of the method, but the original thiamin content of the peanuts may have varied to this extent. The average thiamin value of the four commercial brands of peanut butter was found to be 380 micrograms per 100 grams.

TABLE 1

Summary of Results of Feeding Four Brands of Peanut Butter to Rats as the Sole Source of Thiamin

Daily supplement	Number of animals	Average weight: At weaning	Average weight: At depletion	Average weight: Final weight	Mean gain: In 3 weeks	Mean gain: S.D [1]	Thiamin per 100 grams
SERIES I		*gm.*	*gm.*	*gm.*	*gm.*		*micrograms*
0	9	45	72	60	−12	......	......
Thiamin, 3 μg.	12	44	71	99	26±2.05	7.1	......
Peanut butter, 1.5 gm.							
Sample No. 1	13	44	80	128	48±3.5	12.7	450
Sample No. 2a	13	44	78	119	41±3.2	11.7	350
Sample No. 3	13	45	78	120	42±2.6	9.5	363
Sample No. 4	13	44	80	122	42±2.8	10.2	363
SERIES II							
0	9	42	66	45	−21	......	......
Thiamin, 3 μg.	10	41	68	88	20±2.6	8.1	......
Peanut butter, 1.5 gm.							
Sample No. 2b	13	43	70	103	33±1.6	5.8	324

[1] Standard deviation.

All the values for the thiamin content of peanut butters exceed those for whole-roasted Spanish peanuts (234 micrograms per 100 grams) reported by Booher and Hartzler (1939) and the values for roasted peanuts (180 and 270 micrograms per 100 grams) reported by Munsell (1940). The values reported here are, however, considerably less than those reported by Sherman (1941)—500 to 600 micrograms per 100 grams—for roasted peanuts and peanut butter and by Borsook (1941)—870 micrograms per 100 grams.

It is possible that the low average value of 70 micrograms per 100 grams of peanut butter found by the Georgia workers for 13 commercial brands was due to the chemical method used and is not representative of peanut butters in general. Even their highest value of 130 micrograms per 100 grams is only about one-third of the average value we found.

The small differences in the moisture content of the four samples analyzed are probably related to the temperature and time of roasting as well as the original moisture content of the peanuts. The larger differences in total ash content of the four samples of peanut butter may be related to the amount of added salt. (No. 4 tasted much saltier than the other three.) The three smooth peanut butters made by large commercial concerns have a remarkably uniform calcium content, an average of .034 per cent (Table 2). A part of the calcium of No. 4 may possibly come from the salt instead of the peanuts. The average calcium content of the four peanut butters is .038 per cent.

TABLE 2

Composition of Peanut Butters

Brand	Water	Total ash	Calcium (Ca)	Phosphorus (P)	Iron (Fe)
	pct.	*pct.*	*pct.*	*pct.*	*pct.*
No. 1	1.8	2.89	.032	.413	.00198
No. 2	1.6	3.44	.034	.393	.00186
No. 3	1.1	4.02	.037	.421	.00167
No. 4	1.2	4.17	.048	.391	.00197
Average	1.4	3.63	.038	.404	.00187

The calcium contents of these four commercial peanut butters are only 50 to 70 per cent of the calcium content of peanuts reported by Sherman (1941) and by McCance *et al.* (1936). It is not known if their figures were obtained from analyses of peanuts with or without the skins.

Because our figures for calcium of peanut butter were so much lower than those reported by others for peanuts, the calcium contents of two samples were redetermined by the usual methods used in this laboratory but with the addition of a known weight of calcium salt [$Ca(H_2PO_4)_2$]. The values obtained were the same as previously determined with 97 and 100 per cent recovery of the added calcium.

The phosphorus values are more uniform than the calcium or iron values. The figures for phosphorus are similar to those reported elsewhere for peanuts *(ibid.)* but the average of the peanut butters is slightly higher than that of the peanuts.

The iron contents of Samples 1, 2, and 4 are very similar. That of Sample 3 is about 13 per cent less than the average of the other three. The average iron content (.00187 per cent) of the four peanut butters is slightly less than that which Sherman (1941) and McCance *et al.* (1936) report for peanuts.

Inasmuch as the peanut butters used in this study were made by large commercial concerns in four widely separated areas of the United States, it seems likely that they are representative of peanut butters on the market

and that the figures, for thiamin and calcium especially, more accurately represent the values for average peanut butters than do those reported in previous studies.

Since peanut butter is probably most commonly consumed as a sandwich filling, it is of interest to compare its contribution of thiamin in this combination. Calculating that one tablespoonful of peanut butter, weighing approximately 15 grams, is the usual quantity used with two slices of bread, peanut butter would furnish about 40 per cent of the thiamin in a sandwich made of whole-wheat bread (containing 50 per cent whole-wheat flour) and about 65 per cent of the thiamin in a sandwich made of white bread. If white flour enriched with thiamin were used in making the whole-wheat bread and the white bread, then the peanut butter would furnish in each case approximately 25 and 30 per cent of the total thiamin content of the sandwiches. However, the peanut butter furnishes only one-third to one-fourth the amount that it could supply if much of the thiamin were not destroyed in the roasting process.

Several investigators—Booher and Hartzler (1939), Higgins *et al.* (1941)—have called attention to the relatively large quantities of thiamin in raw peanuts and in the skins and have shown that losses during the roasting process are commonly 78 to 85 per cent. However, since raw peanuts are unpalatable and unsuited for human consumption, it is impractical to recommend the consumption of peanuts in any other than the cooked form. Obviously, the potential values of peanut products, including peanut butter, as economical sources of thiamin in the diet are not being realized at present. It should be possible for the peanut industry and the commercial concerns manufacturing these products to devise ways of making peanuts palatable without serious destruction of their excellent original thiamin content. The American diet has been shown to be universally low in its thiamin content, and steps are being taken to improve this condition by fortification of white flour and by new processes of milling wheat to retain the thiamin and other valuable constituents of the grain. If new methods can be applied in the flour milling industry, doubtless new methods can also be applied in the peanut industry without undue cost to the manufacturers and with nutritional benefit to the consumers.

SUMMARY

Three samples of smooth peanut butter and one of coarsely ground peanut butter made by large commercial concerns in the United States have been assayed biologically for thiamin and analyzed chemically for moisture, total ash, calcium, phosphorus, and iron.

The thiamin contents varied from 324 to 450 micrograms per 100 grams, an average of 380 micrograms per 100 grams.

The three smooth peanut butters contained an average of .034 per cent calcium and the coarsely ground butter contained .048 per cent calcium, an average of .038 per cent for the four samples. The phosphorus contents, which were nearly uniform, averaged .404 per cent. The iron content varied from .00167 to .00198 per cent with an average of .00187 per cent.

Comparison of the data obtained with those in the literature is made. Peanut butter as a source of thiamin is discussed.

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MORTALITY OF MICROORGANISMS DURING PASTEURIZATION OF CUCUMBER PICKLE [1,2]

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During the manufacture of uncured pickle products, controlled pasteurization, 71.1°C.(160°F.) for 20 minutes or 73.9°C.(165°F.) for 15 minutes, must be employed or spoilage of such products will ultimately take place. Previous reports by Etchells (1938), Etchells and Goresline (1940), and Etchells and Ohmer (1941) have repeatedly stressed the importance of this fact. Often active fermentation in improperly pasteurized lots may not become apparent within one month after processing. To illustrate, it has been found that in the manufacture of fresh cucumber sliced pickle and fresh uncured dills, application of the appropriate hot liquor,[3] at approximately 76.7°C.(170°F.), may reduce the numbers of spoilage organisms markedly; and the products may appear to have keeping quality but upon storage may become a total loss owing to the growth of the few organisms which survived.

It is imperative, therefore, that the pasteurizing treatment destroy all microbial life capable of fermenting the liquor. Previous experimental work by Etchells and Goresline (1940) and Etchells and Ohmer (1941) has demonstrated that two groups of organisms are chiefly responsible for spoilage of cucumber pickle. These are (1) acid-forming bacteria and (2) yeasts. The fact that a few organisms of one group or the other and often of both usually survive the application of hot liquor, coupled with their ability to grow at the acid content of the finished uncured pickle products, explains the role these organisms play as a spoilage factor when there is improper pasteurization of such products.

The material to be presented will deal principally with the mortality of strains of acid-forming bacteria and yeasts added to cucumber pickle varying in acid content and subjected to various experimental pasteurizing temperatures.

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[1] Presented at the Technical School for Pickle and Kraut Packers at East Lansing, Michigan, Feb. 17, 18, and 19, 1942.

[2] Agricultural Chemical Research Division Contribution No. 67. Approved for publication as paper No. 140 of the Journal Series of the North Carolina Agricultural Experiment Station.

[3] Hot liquor made up so the two types of pickle had the following final acid and sugar contents: fresh cucumber sliced pickle, 1.4 to 1.6 per cent acetic acid and 16 to 17 degrees Baumé; fresh dill pickle, .7 to .8 per cent acetic acid and no added sugar.

EXPERIMENTAL PROCEDURE

Previously manufactured fresh cucumber pickle was used in the experimental pasteurization studies. This pickle provided a desirable test medium since the slices and liquor had been in contact long enough to attain equilibrium. Furthermore, it was comparatively free from microorganisms; only the heat-resistant, spore-forming types were present and these occurred in such low numbers as not to interfere with the bacteriological analysis. The results (Table 1) of the examination of five representative 25-ounce jars of the same batch of pickle revealed (in addition to the presence of relatively few bacteria and the absence of yeasts) that the acid content ranged from 1.5 to 1.6 per cent acetic acid [4] and 16.5 to 17 degrees Baumé. Also, the ratio of slices to liquor was about five to three by volume.

Two variations in the acid and sugar contents of the original liquor covering the pickle were obtained by pouring off the liquor (250 c.c. taken as the average total amount per jar) and replacing fractions of it with sterile water. In this manner, in addition to using the undiluted liquor, designated as jar treatment A, dilutions of the liquor were made so that approximately one-half and one-quarter of the original amount of liquor remained; these were designated as jar treatments B and C, respectively. An outline of the jar treatments (A, B, and C) including the acidity, Baumé, and pH of the undiluted and diluted liquors, prior to returning them to the slices, is given (Table 2).

TABLE 1

Examination of Representative 25-Ounce Jars of Fresh Cucumber Pickle (1938 Season) Used for Experiments

Jar No	Microorganisms		Acid content [1]	Degrees Baumé	Total volume of pickle	Volume of slices [2]	Volume of liquor
	Bacteria	Yeasts					
	per c.c.	*per c.c.*			*c.c.*	*c.c.*	*c.c.*
1	10	0	1.51	16.5	685	435	250
2	90	0	1.53	17.0	683	436	247
3	0 [3]	0	1.52	16.5	695	425	270
4	0 [3]	0	1.47	16.5	680	425	255
5	0 [3]	0	1.60	17.0	675	428	247
Mean			1.53	16.7	683.6	429.8	253.8

[1] Per cent acetic acid [2] Ratio of slices to liquor, about 5 3 by volume [3] Less than 10 per c c

Five temperatures were employed in the pasteurization studies, 48.9, 54.4, 60.0, 65.6, and 71.1°C. (120, 130, 140, 150, and 160°F.). A certain number of jars were held at each temperature for 15 minutes and promptly cooled with running water. The pasteurizations were carried out by immersing the jars in water in enameled metal tubs (five and one-half gallon capacity) which were heated with two-burner Pyrofax gas stoves. During heating, the water was circulated by a small motor-driven stirrer.

Seven jars were included in each pasteurizing treatment, consisting of duplicate 25-ounce jars for each jar treatment (A, B, and C) and one jar for temperature control. The last was supplied with thermometer inserted

[4] Equivalent to 15 to 16 "grains" acetic acid.

through a hole in the metal cap and insulated from the metal by a cork bored to fit the thermometer. Temperature readings for both water bath and jar contents were taken at 10-minute intervals up to the holding period and then at five-minute intervals.

The test organisms used included one strain of an acid-forming bacterium and two strains of yeast. These were isolated from cucumber fermentations. Inoculations of each lot of pickle to be heated were made from young, vigorous cultures growing in dextrose (.5 per cent) tryptone (.5 per cent) broth (Table 3). The inoculation procedure was as follows: At the time each pasteurizing run was made, the broth cultures were added to the final dilutions of the liquor going to make up jar treatments B and C. The mixtures were then shaken and returned to the slices. In the case of jar treatment A (undiluted), the liquor was poured off and the cultures were added to it, after which the liquor was shaken and returned to the slices. As soon as the cultures were added, the three lots of jars in duplicate were placed in the water bath and heating was begun. Unheated, room-temperature control jars for treatments A, B, and C were likewise inoculated and examined at the start and finish of each pasteurization run with respect to the numbers of organisms present.

TABLE 2

Jar Treatment for Experimental Lots of Pickle With Respect to Dilution of Original Liquor Covering Slices

Treatment	Lot A (full-strength)	Lot B (½ strength)	Lot C (¼ strength)
Amount of original liquor retained per jar	250 c.c.	125 c.c.	62.5 c.c.
Amount of sterile water added to replace liquor	0 c.c.	125 c.c.	187.5 c.c.
Acid content of liquor mixture [1]	1.53 [2]	.76 [2]	.37 [2]
Baumé of liquor mixture [1]	16.7°	8.6°	5.0°
pH of liquor mixture [1]	3.18	3.25	3.44

[1]These observations were made on liquors prior to returning to the slices. [2]Per cent acetic acid.

TABLE 3

Growth of Test Organisms in Dextrose-Tryptone Broth and Amount of Each Culture Added to Each Jar of Pickle

Experimental run	Incubation period for cultures at 35°C (95°F)	Plate count of broth cultures: Acid-former [1]	Plate count of broth cultures: Yeast [2]	Amount of broth culture added per 25-oz. jar: Acid-former [1]	Amount of broth culture added per 25-oz. jar: Yeast [2]
	hr	per c c	per c.c	c c.	c c
1 (Table 4)	72	11,000,000	22,000,000	2	1
2 (Table 5)	72	57,000,000	40,000,000	3	1.5
3 (Table 7)	96	25,000,000	20,000,000	3	2
4 (Table 9)	96	19,000,000	20,000,000	225	225
....	96	....	18,000,000 [3]	......	225 [3]

[1]Acid-former No. V-6 [2]Yeast No. F C [3]Yeast No. V 13.

Note—In experimental runs No 1, 2, and 3, the inoculum per duplicate set of jars was a composite of the amounts indicated. In experimental run No. 4, separate jars (in duplicate) for each of the three test organisms listed were used.

The bacteriological counts for the acid-forming bacteria in the jars of heated pickle as well as the room-temperature controls were made after

shaking the jars and plating dilutions of the liquor on nutritive caseinate agar (Difco) as described previously by Etchells and Goresline (1940). The yeast counts were made in the same manner except that acidified dextrose agar [5] was employed as the plating medium. All plates were incubated 72 hours at 35° C. (95° F.) and counted. Observations as to acidity, sugar content, and pH were made according to methods previously outlined by Etchells and Ohmer (1941).

Some experimental work was carried out to determine the effect of heat upon cultures added to jars of sterilized fresh cucumber slices to which no acid or sugar was added during preparation, the slices being covered with .85 per cent saline. In the first series of such experiments, the regular previously outlined procedure as to cultures, temperatures, and

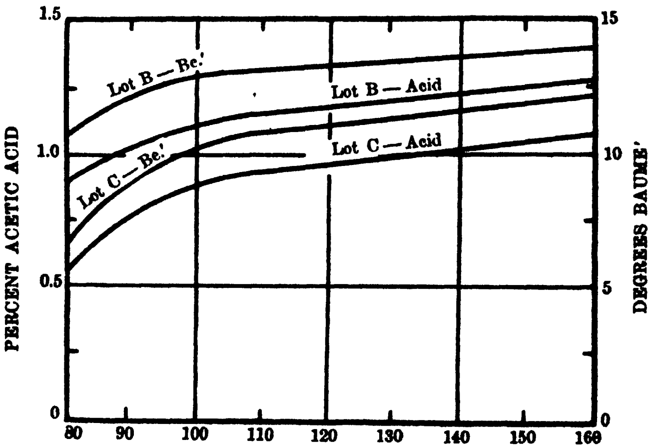

FIG. 1. Increase in acid and sugar contents of the one-half strength (Lot B) and one-quarter strength (Lot C) liquors during heating of 25-ounce jars of pickle to 71.1°C. (160°F.).

holding times was employed. In the second series, a very large inoculum was used (225-c.c. amounts of individual cultures) and the jars were sampled progressively for bacteriological analysis at the times they reached the following temperatures during heating: 48.9, 54.4, 60.0, 65.6, 71.1, and 76.7°C.(120, 130, 140, 150, 160, and 170°F.). Any further variations in the general procedure for certain experiments will be mentioned later.

DISCUSSION OF RESULTS

It is well to point out that during the heating of jars receiving treatments B and C (Table 1) there was an increase in acid and sugar contents of the liquors. This was due to the fact that the sugar and acid contents of the slices were unaltered prior to covering with the diluted experimental liquors, and therefore these constituents were at a higher concentration in the slices than in the liquor. Equalization began when the one-half strength (Lot B) and the one-quarter strength (Lot C) liquors were poured on the slices. The curves presented (Fig. 1) give a reasonable indication as to

[5] Laboratory Manual *(Methods of Analysis of Milk and Its Products)*, Internatl. Assoc. Milk Dealers, Chicago, 1933.

the increase in acid and sugar contents of the liquors for Lots B and C during the heating of the 25-ounce jars of pickle from 26.7 to 71.1°C. (80 to 160°F.). The time required for heating each lot was about 80 minutes. The approximate final values, with respect to acid and sugar contents, reached by jars of Lots B and C during the 80-minute heating interval to 160°F. were as follows: Lot B, 1.26 per cent acetic acid, 14 degrees Baumé; Lot C, 1.07 per cent acetic acid, 12.4 degrees Baumé. At the end of two hours, the values for the unheated room-temperature control lots were essentially the same as for the heated lots.

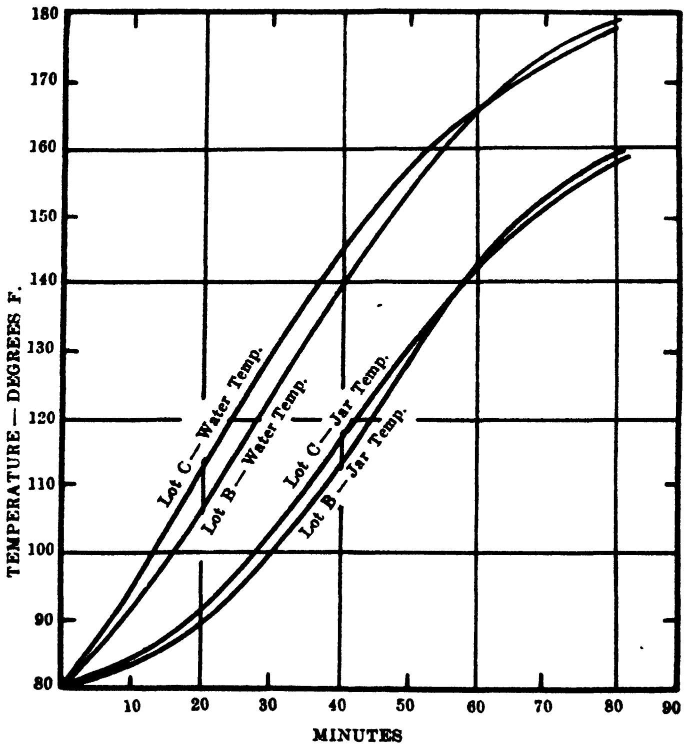

FIG. 2. Rate of heat penetration to center of 25-ounce jars of pickle covered with one-half strength (Lot B) and one-quarter strength (Lot C) liquors during heating to 71.1°C.(160°F.).

The rate of heating for the different lots must also be considered. Curves representing temperature changes in the water bath and jars for Lots B and C (Fig. 2) show that the water bath temperature rose slightly more rapidly for Lot C than Lot B and accordingly the jar temperatures for the two lots were correspondingly different. However, it is evident from these curves that there was no important difference in the rate of heat penetration into the jars of the two lots. A similar experiment involving the use of Lot A jars (full-strength liquor), when corrected for the difference in rate of heating of the water bath, showed a temperature change curve for the jars very similar to those presented for Lots B and C. Therefore, it is concluded that in the pasteurization of the different lots,

any small difference in rate of temperature change in the jar was of no significance so far as the effectiveness of the treatment was concerned.

The results of a preliminary run on the effect of temperatures from 120 to 160°F. for 15 minutes upon inoculated jars of duplicate 25-ounce amounts of pickle are shown (Table 4). In this instance, the initial numbers of acid-forming organisms for the three jar treatments (A, B, and C) ranged from 73,000 to 83,000 per c.c. of liquor, and the numbers of yeasts from 85,000 to 99,000. It will be noted that there is a definite correlation

TABLE 4

Effect Upon Microorganisms of Pasteurizing 25-Ounce Jars of Pickle at 120, 130, 140, 150, and 160°F. for 15 Minutes (First Run)

Pasteurization treatment	Jar treatment	Survival plate count	
		Acid-forming bacteria	Yeasts
°F.		per c.c.	per c.c
Room-temperature controls[1]	A	73,000	91,000
	B	74,000	85,500
	C	81,000	99,000
120	A	0[2]	0[2]
	B	100	50
	C	1,100	5,000
130	A	0[3]	0[3]
	B	0[3]	200
	C	300	550
140	A	0[3]	0[3]
	B	0[3]	0[3]
	C	100	0[3]
150	A	0[4]	0[4]
	B	0[4]	0[4]
	C	30	70
160	A	0[4]	0[4]
	B	0[4]	0[4]
	C	0[4]	0[4]
Room-temperature controls[5]	A	0[2]	6,000
	B	4,500	21,500
	C	11,000	43,500

[1] At start of experiment (9:30 a.m.). [2] Less than 1,000 per c.c. [3] Less than 100 per c.c. [4] Less than 10 per c.c. [5] At end of experiment (12 00 noon)

between the strength of the liquor used and the destruction of both acid-forming bacteria and yeasts. Also, it will be noted that the 160°F. pasteurizing temperature was successful in reducing the organisms in all three treatments to insignificant numbers (less than 10 per c.c.). Since the plating dilutions were not low enough for Lot A pasteurized at 120, 130, and 140°F. and Lot B at 130 and 140°F., no exact estimate can be given as to the numbers of surviving organisms at these pasteurizing temperatures. However, it appears obvious that with the inoculum first used, the mortality in the A and B lots of pickle was rapid even at the lower temperatures. Furthermore, it is well to point out that there was a

marked reduction from the initial counts of organisms brought about by holding the control jars at room temperature for two and one-half hours. The most marked reduction was suffered by the acid-forming bacteria in the full-strength liquor (A). Here the initial count of 73,000 per c.c. was reduced to less than 1,000 per c.c. The yeast count in this lot was reduced from 91,000 to 6,000 per c.c. The initial counts of one-half strength lot (B) and one-quarter lot (C) likewise showed decreases, corresponding to the strength of the liquors. In general, it would appear that the yeasts

TABLE 5

Effect Upon Microorganisms of Pasteurizing 25-Ounce Jars of Pickle at 120, 130, 140, 150, and 160°F. for 15 Minutes (Second Run)

Pasteurization treatment	Jar treatment	Survival plate count	
		Acid-forming bacteria	Yeasts
°F.		*per c.c.*	*per c.c.*
Room-temperature controls[1]	A	650,000	150,000
	B	670,000	180,000
	C	720,000	190,000
120	A	550	140
	B	16,000	11,300
	C	64,000	20,800
130	A	Sp.[2]	0[3]
	B	470	450
	C	6,800	1,600
140	A	0[3]	0[3]
	B	270	50
	C	500	10
150	A	0[4]	0[4]
	B	10	0[4]
	C	25	20
160	A	0[4]	0[4]
	B	0[4]	0[4]
	C	0[4]	0[4]
Room-temperature controls[5]	A	8,000	45,000
	B	120,000	63,000
	C	370,000	67,000

[1] At start of experiment (11 30 a m) [2] Less than 10 per c.c [3] Less than two per c.c [4] Spreaders on 1-10 dilutions, no test organisms on 1 100 dilutions [5] At end of experiment (1:15 p.m).

were more resistant than the acid-forming bacteria with respect to the action of the liquor alone.

The numbers of organisms from heavier inoculations which survived pasteurizing temperatures of 120 to 160°F. for 15 minutes in pickle of different liquor strengths are shown (Table 5). In this run, about a ninefold increase in acid-forming bacteria and nearly a twofold increase in yeasts over the numbers added in the preliminary run, were employed. Also, lower plating dilutions were used. The results show that even with the heavier inoculations the 160°F. pasteurization procedure was sufficient to reduce the acid-forming bacteria and yeasts in all three jar treatments

to insignificant numbers (less than two per c.c.). One point of interest in connection with the use of a larger number of organisms is that at temperatures below 150°F. a greater number survived the respective pasteurizations as compared with the surviving numbers in the first run (Table 4). Further examination of Table 5 reveals the previously mentioned relationship between the liquor strength (Lots A, B, and C) and the numbers of acid-forming bacteria and yeasts surviving the respective pasteurizing treatments up to the 160°F. treatment. Here it will be noted that in general the mortality of the two groups of organisms within any one pasteurization treatment is greatest in the more-acid liquor (Lot A) and correspondingly lower in less-acid liquors (Lots B and C). When the initial numbers of both groups of organisms are considered, it would ap-

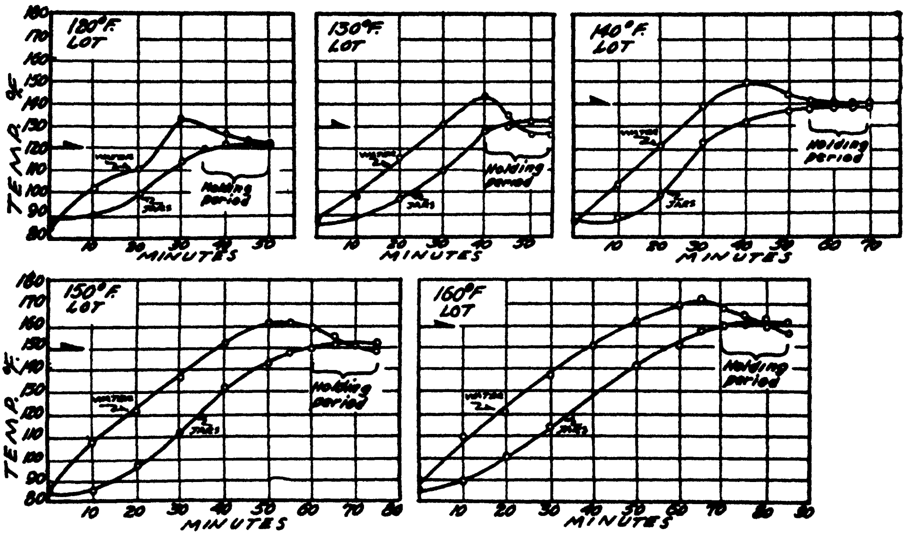

FIG. 3. Rate of heat penetration to center of 25-ounce jars of pickle during pasteurization treatments of 48.9, 54.4, 60.0, 65.6, and 71.1°C. (120, 130, 140, 150, and 160°F.) for 15 minutes.

pear from the percentage of surviving organisms, that the acid-forming bacteria are less resistant to pasteurization treatments under 160°F. than are the yeasts. In attempting to evaluate the data concerning the mortality of the test organisms during the various pasteurizing treatments, including the three liquor strengths used, one must bear in mind that even under room-temperature conditions a goodly proportion of the organisms would have been destroyed, owing presumably to the action of the acid. The survival plate counts for the room-temperature controls (Table 5) made at the conclusion of the pasteurizations bear out this relationship. These counts indicate that during the one hour and 45 minute interval, the acid-forming bacteria were more susceptible to the full-strength liquor than were the yeasts. Curves are given (Fig. 3) showing the rate of heat penetration to the center of 25-ounce jars for the various pasteurized lots previously discussed in connection with Tables 4 and 5.

The numbers of surviving organisms in the room-temperature control jars plated at intervals up to four days are further presented (Table 6). The results with respect to numbers of acid-forming bacteria and yeasts present in the three lots (A, B, and C) show that by the end of four days only a small proportion of the original inoculum survived.

The material presented so far has dealt with the effect of pasteurization on inoculated cucumber pickle containing added acid and sugar. Another series of pasteurizations was made with inoculated pickle containing no added acid or sugar, using essentially the same previously described procedure. The results with respect to numbers of organisms surviving the various heat treatments are presented (Table 7). In this particular run, 210,000 acid-forming bacteria and 155,000 yeasts per c.c. of liquor were

TABLE 6

Bacteriological Analysis of Duplicate 25-Ounce Jars of Inoculated Pickle, Held at Room Temperature (85° F.)

Jar treatment	Time interval	Acid-forming bacteria	Yeasts
		per c.c.	*per c.c.*
A	Initial	650,000	150,000
B	Initial	670,000	180,000
C	Initial	720,000	190,000
A	1¾ hr.	8,000	45,000
B	1¾ hr.	120,000	63,000
C	1¾ hr.	370,000	67,000
A	4½ hr.	4,000	24,000
B	4½ hr.	120,000	24,000
C	4½ hr.	350,000	56,000
A	24 hr.	10,000	24,000
B	24 hr.	75,000	23,000
C	24 hr.	340,000	30,000
A	4 days	5,000	800
B	4 days	17,000	300
C	4 days	87,000	......

added at the start. The survival counts with respect to acid-forming bacteria show progressive sharp declines as higher pasteurizing temperatures were used, up to 150°F., this temperature applied for 15 minutes being successful in reducing the count to less than two per c.c. The results for the yeasts show a similar trend. It will be noted that both groups of organisms in the room-temperature controls slightly increased in numbers during the one and one-quarter hours required during the pasteurizations. The growth in similar material (in absence of added acid and sugar) over a longer period of time at room temperature is presented (Table 8). It will be seen that there was active fermentation by both groups of organisms using for their growth requirements only the naturally occurring constituents of the sterilized fresh cucumber slices. This activity was in direct contrast to the behavior of similarly treated lots to which sugar and acid had been added (compare Tables 8 and 6).

TABLE 7

Effect Upon Added Microorganisms of Pasteurizing 25-Ounce Jars of Sterilized, Fresh Cucumber Slices Containing No Added Vinegar or Sugar at 120, 130, 140, 150, and 160°F. for 15 Minutes

Pasteurization treatment	Survival plate count	
	Acid-forming bacteria	Yeasts
°F.	per c.c.	per c.c.
Unheated control[1]	210,000	155,000
120	47,000	100,000
130	450	380
140	20	0[2]
150	0[3]	0[3]
160	0[3]	0[3]
Unheated control[4]	260,000	186,000

[1] Room-temperature control at start of experiment (11:45 a.m.). [2] Less than 10 per c.c. [3] Less than two per c.c. [4] Room-temperature control at end of experiment (1:00 p.m.).

TABLE 8

Growth of Inoculated Microorganisms at Room Temperature (85°F.) in 25-Ounce Jars of Sterilized, Fresh Cucumber Slices Containing No Added Vinegar or Sugar

Time interval	Acid-forming bacteria	Yeasts
	per c.c.	per c.c.
Initial	770,000	190,000
1¾ hr.	1,500,000	100,000
4½ hr.	7,760,000	700,000
24 hr.	18,000,000	12,500,000
4 days	16,000,000	5,000,000

TABLE 9

Effect of Exposure to Temperatures of 120, 130, 140, 150, 160, and 170°F. Upon 225-Cubic Centimeter Amounts of Individual Cultures Added to 25-Ounce Jars Sterilized, Fresh Cucumber Slices

Maximum temperature attained	Survival plate count		
	Acid-former (No. V-6)	Yeast (No. F C)	Yeast (No. V-13)
°F.	per c.c.	per c.c.	per c.c.
Unheated controls[1]	19,800,000	18,000,000	20,000,000
120	5,200,000	1,800,000	14,000,000
130	10,000	16,000	3,800,000
140	0[2]	0[2]	600
150	0[2]	0[2]	50
160	0[2]	0[2]	0[2]
170	0[2]	0[2]	0[2]
Unheated controls[3]	22,000,000	19,000,000	24,000,000

[1] Room-temperature control at start of experiment (3:00 p.m.). [2] Less than 50 per c.c. [3] Room-temperature control at end of experiment (5:00 p.m.).

A second experiment using sterilized fresh cucumber slices was set up to test the effect of exposures to temperature ranging from 120 to 170°F. upon a very large inoculum of three organisms tested individually. Inoculation of the jars was in duplicate, using separate 225-c.c. amounts of broth culture for each of the three organisms (one acid-former and two yeasts). No holding time was employed; instead, the samples were removed and plated when the jars attained the desired temperature. An attempt was made to keep the inoculum approximately the same with respect to numbers of cells of each organism used. This would aid in determining any differences between organisms with regard to heat resistance; the results of this experiment are shown (Table 9). It will be noted that the reduction in numbers was most rapid in the case of the acid-forming bacteria, the count being reduced from 19,800,000 per c.c. to less than 10 per c.c. by the time the 140°F. temperature was reached. It must be pointed out, however, that in the case of the acid-forming bacterium, the four-day-old culture medium contained a considerable amount of lactic acid, probably in the neighborhood of .8 to 1 per cent, and this presumably contributed in part to the rapid destruction of the bacteria. The results for the two strains of yeasts used showed that one strain (No. V-13) survived the 150°F. maximum temperature while the other (No. F C) may be considered to have been killed by exposure to 140°F. The room-temperature controls showed slight increases for all three organisms during the two hours required for the experiment.

SUMMARY AND CONCLUSION

The results of a series of experiments dealing with the mortality of microorganisms during the pasteurization of cucumber pickle have been presented. Pasteurization procedures using temperatures of 120, 130, 140, 150, and 160°F. applied for 15 minutes were carried out on three lots of inoculated cucumber pickle which varied with respect to acid and sugar contents of the liquor (full-strength, one-half strength, and one-quarter strength). One strain of an acid-forming bacterium and two strains of yeast were used as the test organisms.

In general, the results show that increasing pasteurizing temperatures beginning with 120°F. brought about corresponding decreases in the numbers of surviving organisms up to 160°F. The latter pasteurizing temperature was sufficient to destroy both acid-forming bacteria and yeasts in all liquor treatments used irrespective of the quantity of inoculum employed. Furthermore, it was noted that with the lower temperature treatments (120 and 130°F.) there was a definite correlation between the number of surviving acid-forming bacteria and yeasts and the acid contents of the three liquors used. Results for the 140 and 150°F. treatments indicated that the organisms added as inoculum were killed in the most-acid liquor, that some survived in the two less-acid liquors, and that the numbers surviving in these were about equal. In addition, the results show that with the inoculated, unpasteurized lots (room-temperature controls) a marked reduction in the number of surviving organisms occurred within one and three-quarters to two and one-half hours, the time required to complete a series

of pasteurization treatments. This effect is due presumably to the acid content of the liquors.

ACKNOWLEDGMENT

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ACIDIFIED VEGETABLE JUICE BLENDS

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Interest in the use of fruit and vegetable juices in various forms has been increasing rapidly. Until a relatively few years ago the only juices available were grape juice and cider during season. Tomato juice and sauerkraut juice were among the first vegetable juices made available. The great difficulty in preparing good carrot, celery, or other vegetable juices of high pH is that the high temperature and long period necessary for sterilization causes undesirable changes in flavor and texture of the juice. If a product of good flavor can be prepared which can be preserved by flash pasteurization, a superior product will be obtained. The subject of vegetable juices, particularly the advantages of acidification in producing superior blends, has recently been reviewed by Marsh (1942).

Walsh and Tressler (1936) prepared a pleasing clarified juice and Beattie and Pederson (1939) prepared an unclarified juice from rhubarb.

Cruess, Thomas, and Celmer (1937) acidified celery juice from blanched celery to .4 per cent citric acid and noted that the acidified juice did not coagulate as badly and had a more pleasing flavor than the nonacidified juice. Cruess and Yerman (1937) found blanching of celery necessary for inhibition of enzyme action. They acidified to .5 per cent citric acid. Cruess and Celmer (1938) concluded that celery juice acidified to .4 per cent citric acid, pH 3.94, was too acid but that juice acidified to .3 per cent acid, pH 4.14, was not too tart and could be processed safely at 100°C.(212°F.).

Recently Cruess and Chong (1941) suggested a blend of carrot juice with orange juice, the acidity being adjusted to about pH 4.05. Lachele (1938) extracted juices from carrots, beets, and celery with a Schwarz disintegrator. Turner (1939) obtained carrot juice from blanched carrots by means of an hydraulic press. Marsh (1938) studied the buffering action of vegetables using hydrochloric, citric, and acetic acid in establishing pH curves for these various acids. He noted loss in acidity during heating that could be explained only on the basis of adsorption by the vegetable tissue.

The above acidified blends were all prepared with solutions of the acid studied except a few prepared with lemon juice. A blend of tomato juice with sauerkraut juice was prepared commercially several years ago, the improvement in flavor from the addition of acid and salt being the main feature of the blend.

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[1] Preliminary work on this project was carried out by the senior author in co-operation with G. L. Marsh at the Fruit Products Division of the University of California. The authors wish to express appreciation to Mr. Marsh and D. K. Tressler for helpful criticisms, and to C. B. Sayre for advice in regard to varieties of vegetables used in these studies.

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Various vegetable juices have been prepared and sold either as fresh juice or as processed juice. Recently a number of vegetable juice blends have been prepared, usually containing several vegetable juices with tomato juice as the major ingredient. Any blend of juices, prepared from vegetables, will have certain nutritive qualities that would make it commendable to the consuming public. Such juices should also be pleasing in appearance as well as in flavor and aroma and should have characteristic vegetable flavor. In addition, the product should be made available at a reasonable cost.

It is generally known that the acid fruits and vegetables can be processed at lower temperatures than the slightly acid or neutral foods. Bigelow and Cathcart (1921) drew up an arbitrary scale of foods in relation to hydrogen-ion concentration and stated "In general, the lower the pH value of foods, or, in other words, the greater the hydrogen ion concentration, the lower the process necessary for sterilization." This thought was further developed in a paper by Bigelow and Cameron (1932) in which the upper limit of the acid range for acid foods was placed at pH 4.5. In a later paper from their laboratory, written by Cameron and Esty (1940), a separate acid grouping of food with pH values between 3.7 and 4.5 was discussed. Occasionally spoilage by anaerobic thermophiles was noted in the group of foods. Pederson (1929), in a study of tomato products, isolated numerous nonsporeforming bacteria all of which could be controlled normally by processes of 82.2°C.(180°F.). On the other hand, Berry (1933) isolated a sporeforming bacterium from tomato juice capable of growing slowly in juice at pH 4.2, but the organism could not be grown in more acid juices. This inability of the organisms to grow in the more acid juices was later observed by one of the authors.

In view of these observations, it was felt that if vegetable juices, which are ordinarily very slightly acid and therefore require high temperature processing, could be made more acid, that is, changed to a hydrogen-ion concentration of about pH 4 to 4.1 so that sporeforming bacteria could not grow, such juice could be processed at considerably lower temperatures. Such acidification may be accomplished with mineral acids, such as hydrochloric or phosphoric, or with organic acids, such as lactic, acetic, or other food acid. It would be preferable in adjusting the acidity to use a vegetable juice containing one of these acids rather than a pure acid solution. Sauerkraut juice, a vegetable juice, contains lactic as well as some acetic acid and in addition the normal nutritive qualities of sauerkraut. An objection to its use for acidification seemed to be the possibility that because of its relatively low acidity and its buffering action, the desired hydrogen-ion concentration of pH 4 to 4.1 in a blend might not be attainable without the addition of so much sauerkraut juice that the flavor of the original vegetable would not be retained. Previous workers noted an improvement in flavor by acidification as well as less coagulation of colloidal material. It would further seem that the vitamins, particularly ascorbic acid, might not be destroyed as readily in that the juice is more acid and, since lower processing temperatures can be used, the container could be filled hot and full, thus reducing the amount of air present.

Blends of various vegetable juices with sauerkraut juice surprisingly produced juices far more palatable than similar blends acidified with hydrochloric, lactic, acetic, or phosphoric acid, even though the latter were salted as much as the sauerkraut blends. In fact, the blends of juices with sauerkraut juice were considered superior to the pure juices by everyone who sampled them. Among these were individuals who did not like sauerkraut as such.

PREPARATION OF JUICE

It is a simpler procedure to extract juice from soft fruits or vegetables than from hard ones, such as carrots, beets, turnips, and celery. Some juice may be obtained by grinding the vegetables and pressing in a hydraulic press, but the product as well as the yield is unsatisfactory in that too little of the pulpy portions are obtained to give desired vegetable flavor. A satisfactory yield of pulpy juice can be obtained by use of small juicers. The "RYP" used in these studies grates the vegetable and separates the juice by centrifugal force. On a larger scale, vegetables were ground in a hammer mill and pressed in a Chisholm Ryder continuous press. It is essential to macerate the vegetables to release the juice from nearly all of the cells. The extracted juice should be blended and processed immediately after extraction in order to avoid excessive enzyme activity. If a blend cannot be prepared immediately, the extracted juice should be heated to 180°F. and cooled.

ACIDIFICATION OF VEGETABLE JUICES

In order to determine the amount of acid required to adjust the hydrogen-ion concentration to definite pH values, vegetable juices were prepared from various lots of celery, carrots, beets, onions, turnips, rutabagas, and red cabbage. To carefully measured aliquots of these were added measured amounts of the various acids, that is, hydrochloric, phosphoric, lactic, and the acids of rhubarb and of sauerkraut juices. The hydrogen-ion concentration was determined with each addition of acid. The juices were also titrated with sodium hydroxide to adjust the hydrogen-ion concentration to about pH 8.1. Results when plotted show typical buffer curves (Figs. 1 to 6). From these one can calculate the amount of any acid needed to change the hydrogen-ion concentration to any desired pH.

The same types of curves are obtained by the addition of acids to each vegetable juice and they differ only in the amount of acid necessary to produce a definite change in hydrogen-ion concentration (Figs. 1 to 6). The amounts of acid required to obtain a definite hydrogen-ion concentration in different samples of one vegetable juice are as variable as for the different vegetable juices (Figs. 1, 3, and 6). In other words, from 19.5 to 35 c.c. of acid were required to adjust the hydrogen-ion concentration of different samples of celery juice to pH 4, from 21 to 83 c.c. for different samples of carrot juice, but only 27 to 53 c.c. for different samples of the other vegetable juices. Cabbage and carrots were found to be among the more highly buffered. March (1938) found peas to be highly buffered.

On the basis of normality, hydrochloric acid is the most effective (Figs. 4 and 5) followed by lactic and phosphoric acids. At the higher hydrogen-ion concentrations the phosphoric acid curves cross the lactic acid

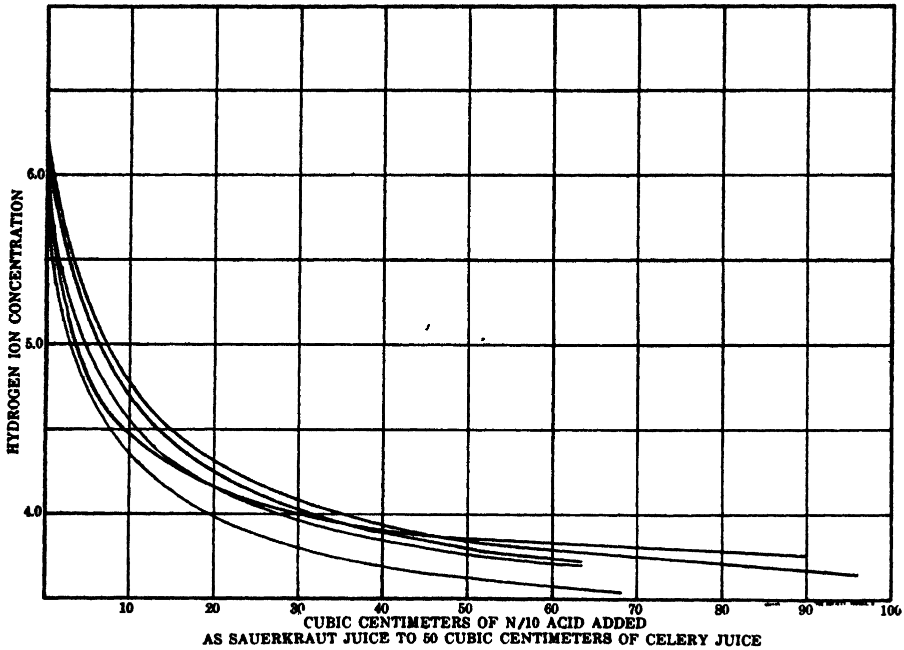

FIG. 1. Comparative effect of sauerkraut juice upon hydrogen-ion concentration of five samples of celery juice.

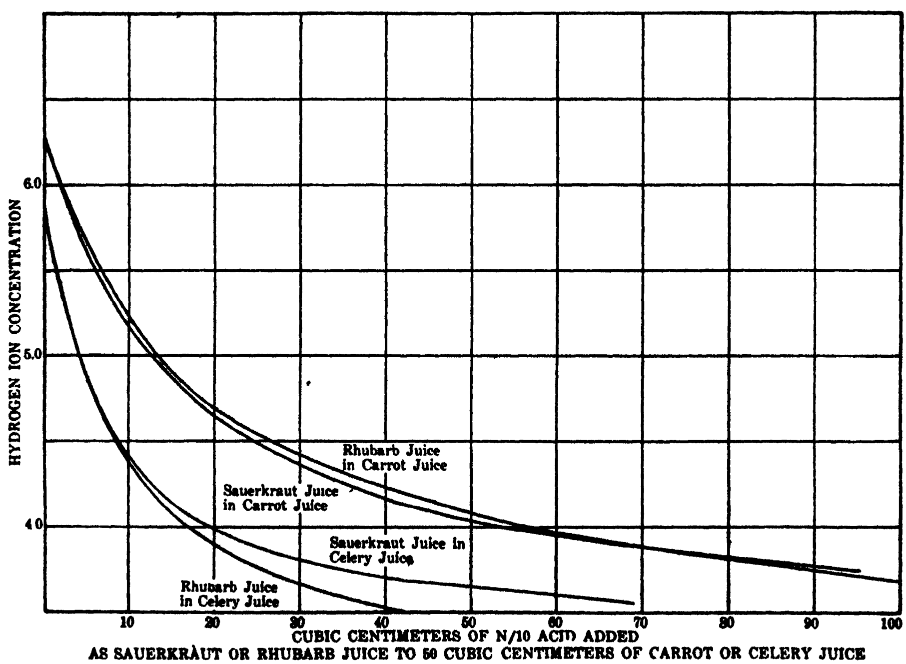

FIG. 2. Comparative effect of acid of rhubarb and sauerkraut juices upon hydrogen-ion concentration of carrot juice and celery juice. Rhubarb juice pH 2.78, normality .254; sauerkraut juice pH 3.18, normality .136.

curves. This is readily accounted for in that these solutions have pH values of about 2.12 and 2.51, respectively. If activity is calculated on the basis of per cent of acid, it is found that the lactic acid has a lesser effect than phosphoric acid. Sauerkraut juice calculated as lactic acid has the least effect of the various acid solutions. This of course is natural since the hydrogen-ion concentration was pH 3.18 and it was highly buffered in itself. Sauerkraut contains acetic acid in proportions of about one to four of lactic.

Individually, several samples of celery juice have varied in pH from 5.96 to 6.11. Comparison of five samples (Fig. 1) show that four of them required a variation of 28 to 35 c.c. of N/10 acid per 50 c.c. of juice to produce a solution of pH 4. The fifth was more poorly buffered in that only 19.5 c.c. of N/10 acid was required to bring about the same change in pH.

Several varieties of both blanched and unblanched celery were used to make juices, the former giving higher yields than the latter. Juices from unblanched Tendergreen, Irondequoit, and Pascal varieties were very desirable. Differences were noted among varieties as to rapidity of darkening and acquiring of bitterness after extraction and before processing, but further variety studies should be made. All varieties of all vegetables should be processed as quickly as possible to prevent enzyme action. If so handled the flavor is more typical of the vegetable than is the flavor of juice extracted after blanching the vegetable.

One sample of celery and one of carrot were titrated with rhubarb juice as well as sauerkraut juice. The rhubarb juice was equivalent to .254 N acid as oxalic, and had a pH of 2.78. In spite of its high acidity, on the basis of normality results were almost identical with those obtained with sauerkraut juice (Fig. 2). The flavors of the celery and carrot juices were almost entirely masked by the rhubarb juice so that little of the original vegetable flavor remained. This was not true of blends with sauerkraut juice.

Carrot juices (Fig. 3) varied considerably in the amount of acid required to change the hydrogen-ion concentration to any definite value. One sample required only 21 c.c. of sauerkraut juice while another required 83 c.c. for 50 c.c. of carrot juice to produce a pH of 4. Unfortunately the sample of carrot used for comparison of acids (Fig. 4) was one of the most poorly buffered. Carrots were found to be the most difficult to extract with satisfactory yield of juice and very fine maceration before pressing was necessary.

Among the other vegetables studied, that is, rutabagas, beets, white turnips, onions, and red cabbage, excepting cabbage little variation was found in their buffer action. The amount of acid from sauerkraut juice required to change the pH to 4 for these vegetables varied from only 27 to 53 c.c. (Fig. 6). Results with lactic, hydrochloric, or phosphoric acid were comparative. The pH of the original juices varied from 5.5 to 6.38. Beet, onion, or white turnip juice with sauerkraut juice were pleasing and all blended well with tomato and celery juices. None of these three could be used in such blends in very high concentrations. Rutabaga juice was very strong in flavor. Red cabbage was flat and tasteless.

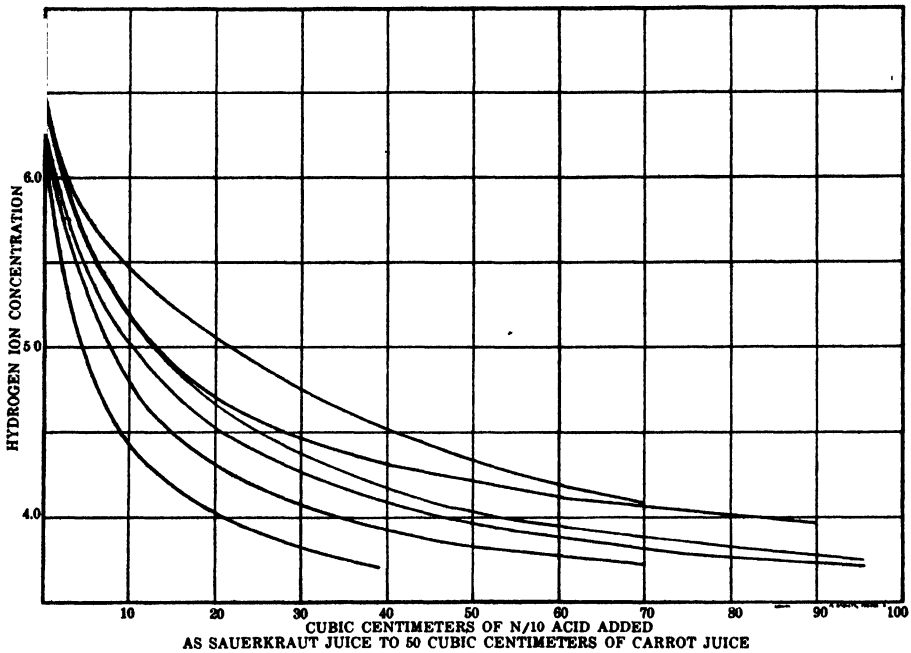

FIG. 3. Comparative effect of sauerkraut juice upon hydrogen-ion concentration of six samples of carrot juice.

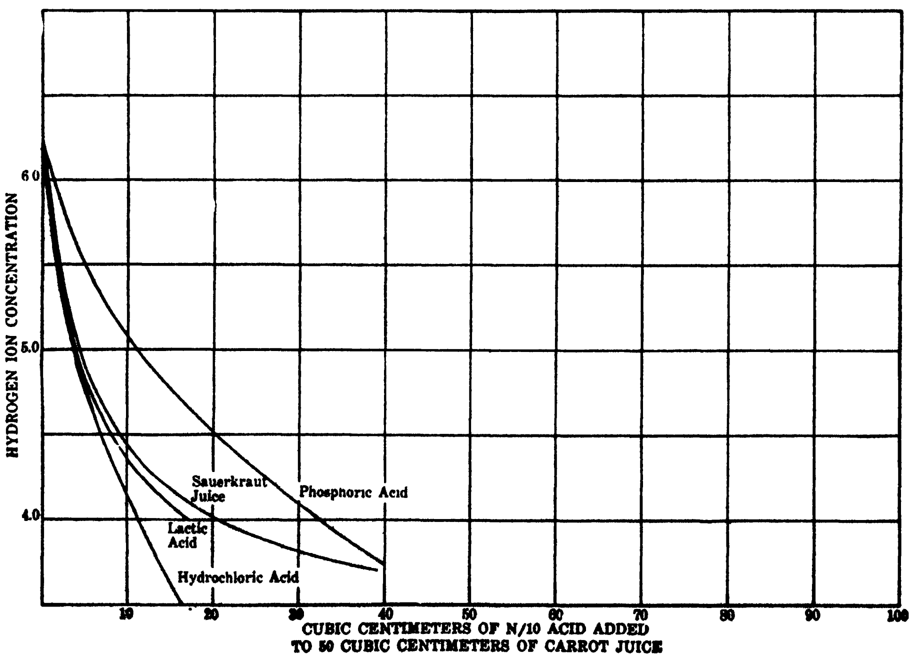

FIG. 4. Comparative effect of hydrochloric, lactic, and phosphoric acids and acid of sauerkraut juice upon the hydrogen-ion concentration of carrot juice.

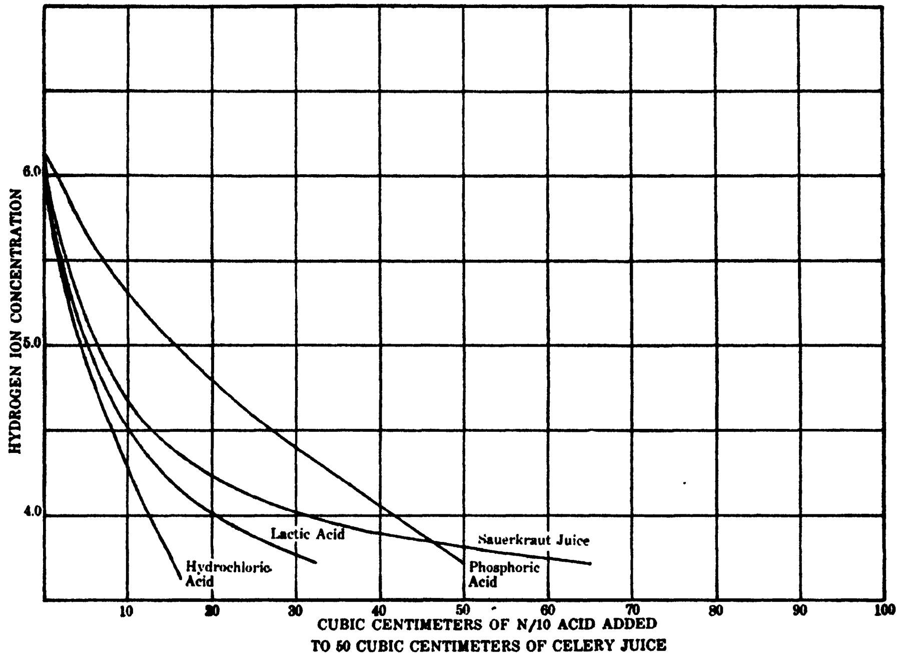

FIG. 5. Comparative effect of hydrochloric, lactic, and phosphoric acids and acid of sauerkraut juice upon the hydrogen-ion concentration of Pascal celery juice.

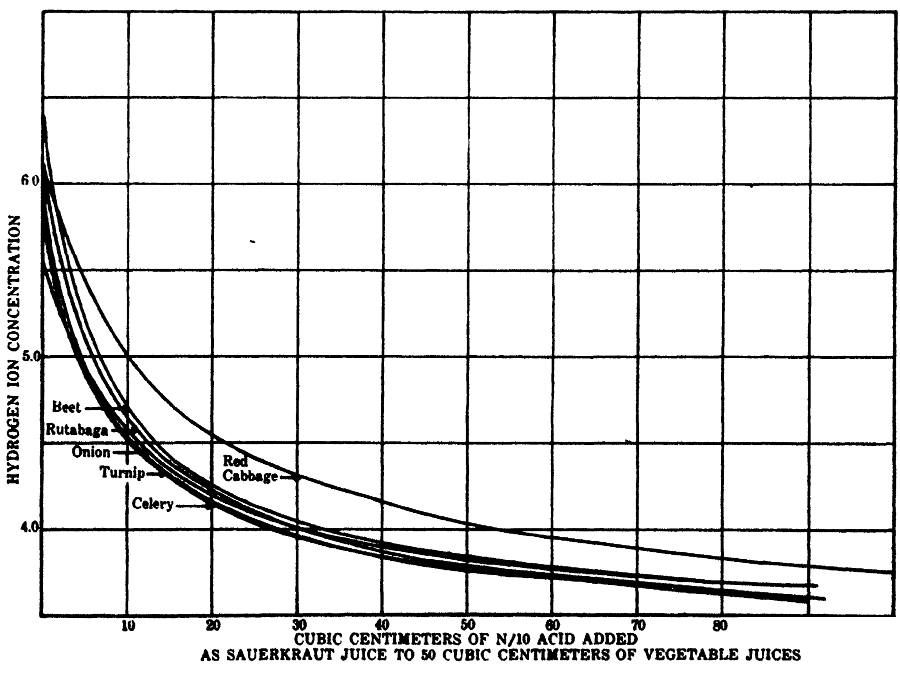

FIG. 6. Comparative effect of acid of sauerkraut juice upon the hydrogen-ion concentration of six vegetable juices.

Excepting these latter, any of the juices blended well with tomato juice in many proportions. Ordinarily the better blends prepared contained about two-thirds or more tomato juice. Beets were particularly valuable in adding color as well as flavor but the amount added had to be small, one to two per cent. Onion juice was also found desirable in small quantities, as was turnip juice.

In pasteurization of these products, bacteriological studies have shown that the vegetative microorganisms present are all killed with flash pasteurization temperatures below 180°F. Deaerating at 14 to 16 mm. and flash heating at temperatures of 180°F. followed by filling into cans or heated bottles has proved to be a satisfactory process for these blends. This procedure is described in greater detail by Pederson and Tressler (1938). The flash pasteurized juices of course were superior in flavor to those processed by pressure sterilization.

DISCUSSION

In the blending of any of these juices with acid, regardless of the acid used, the importance of accurate control cannot be overemphasized. From a study of the data presented, it is impossible to predict the amounts of acid, either mineral or organic acid, necessary to adjust the hydrogen-ion concentration to any definite pH. This is of course due to the difference in buffer content of the various samples of juices. This is further complicated by the varying buffer content of different samples of kraut or rhubarb juice as well as the varying amount of acid present in such juices. Therefore, in the preparation of such blends each lot of vegetable juice will have to be adjusted with each lot of acid before the actual blend is prepared.

It is desirable to obtain a high acid sauerkraut juice, 1.5 per cent or higher, for blending purposes in order that as little may be used in a blend as possible. If it is desired that less juice be added to the blends, the sauerkraut juice may be concentrated by any one of several procedures. Although this may decrease the volume of juice added, it does not decrease the buffer content or the kraut flavor. The intensity of the kraut flavor may be decreased considerably by treating with charcoal but this is not necessarily desirable.

SUMMARY

Juices were extracted from celery, carrots, beets, onions, turnips, rutabagas, and red cabbage. Pleasing blends of these with sauerkraut juice were prepared. When acidified to pH 4 these could be flash pasteurized at 180°F. with little loss of the characteristic vegetable flavor. Such blends of carrot, celery, beet, onion, and turnip juice further blended well with tomato juice, resulting in various pleasing vegetable beverages.

Mineral acids, such as hydrochloric and phosphoric, and organic acids, such as lactic and oxalic in rhubarb juices, were used to acidify the various vegetable juices. Although all except oxalic acid in rhubarb juice could be used in smaller quantities than sauerkraut juice to change the hydrogen-ion concentration of the blend, none of them gave a blend that was as satisfactory as those with sauerkraut juice.

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GROWTH OF A FOOD-POISONING STRAIN OF STAPHYLOCOCCUS EXPERIMENTALLY INOCULATED INTO CANNED FOODS [1]

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Since the staphylococcus is the most common etiological agent in food poisoning it is important that more be known about its growth in our common foods. Although the canning of foods involves time and temperature relationships which exclude this organism, nevertheless, cans of food are often opened and the contents exposed to time and temperature conditions which are favorable to the growth of staphylococci. The widespread occurrence of this organism affords it access to food exposed to air. The purpose of this investigation, therefore, was to study the growth of staphylococci in experimentally inoculated cans of food. By such a procedure the organisms were studied in pure culture without competition with other naturally occurring, contaminating microorganisms.

The canned foods studied in these experiments include corn, peas, asparagus, spinach, string beans, tomato juice, peaches, shrimp, and salmon. These foods were chosen since they represent a cross section of the different types of food on the market; for example, peas and corn are low-acid products; asparagus, spinach, and string beans are semiacid products; whereas tomato juice and peaches are acid products. Shrimp and salmon are examples of sea food the chemical composition of which is sufficiently different from fruits and vegetables to warrant study. Studies on corned beef, roast beef, and potted meat as an example of another type of food will be reported later.

EXPERIMENTAL PROCEDURE AND RESULTS

In all experiments *Staphylococcus aureus*, Strain 161, was used. This strain was isolated from an outbreak of food poisoning reported by Dack, Bowman, and Harger (1935). Since its isolation this strain has consistently produced enterotoxin in repeated tests on human volunteers, monkeys, and kittens. The organisms were grown in veal infusion broth for six to 24 hours and the broth culture, or a dilution thereof, inoculated directly into cans of different foods.

Before inoculation of a can with staphylococci the top of the can was flooded with alcohol and the alcohol burned off. With a sterile iron tip a hole was made in this end of the can and inoculation was done by means of a sterile hypodermic syringe containing the broth culture. When a can was too full, some of the fluid contents was aspirated before inoculation. The opening was resealed with solder. Contamination was seldom encountered with this technique.

[1] This work was aided by a grant from the National Canners Association.

TABLE I

ndividual Counts on Each Food Inoculated With Staphylococcus aureus, Strain 161

Series	Canned food	Number of organisms inoculated per ml. of fluid contents (Size of inoculum = 1 ml.)	Number of organisms per ml. of fluid contents after incubation for									
			2 days		7 days		14 days		30 days		60 days	
			22°C.	37°C.	22°C.	37°C.	22°C.	37°C.	22°C.	37°C.	22°C.	37°C.
1	Asparagus..........	51×10^{4}	3×10^{7}	264×10^{4}	175×10^{5}	2×10^{4}	*	*	*	*	0	6
2	Asparagus..........	217×10^{4}	288×10^{5}	16×10^{4}	1×10^{6}	3×10^{5}	*	*	18×10^{4}	7×10^{3}	*	2
3	Asparagus..........	156×10^{4}	342×10^{4}	202×10^{4}	46×10^{5}	12×10^{4}	*	*	6×10^{4}	0	*	1
4	Asparagus..........	73×10^{4}	4×10^{6}	117×10^{4}	88×10^{4}	15×10^{4}	*	*	24×10^{4}	0	*	Con.**
5	Asparagus..........	1×10^{6}	218×10^{5}	88×10^{6}	142×10^{6}	$<10^{4}$	*	1×10^{3}	81×10^{6}	10	7×10^{2}	0
1	Spinach..............	3×10^{6}	6×10^{7}	14×10^{6}	2×10^{6}	6×10^{5}	27×10^{3}	219×10^{2}	$<10^{3}$	$<10^{3}$	<10	2
2	Spinach..............	1×10^{5}	35×10^{4}	19×10^{4}	133×10^{3}	2×10^{3}	25×10^{4}	97×10^{2}	7×10^{5}	$<10^{2}$	65×10^{4}	1
3	Spinach..............	3×10^{6}	37×10^{5}	48×10^{4}	59×10^{5}	148×10^{3}	22×10^{3}	1×10^{3}	112×10^{3}	276×10^{3}	61×10^{3}	126×10^{3}
4	Spinach..............	5	11×10^{5}	$>10^{4}$	293×10^{5}	244×10^{5}	52×10^{5}	191×10^{6}	104×10^{6}	213×10^{4}	23×10^{5}	11×10^{6}
1	String beans.......	38×10^{4}	16×10^{4}	13×10^{5}	1×10^{5}	3×10^{5}	*	*	*	*	*	*
2	String beans.......	168×10^{4}	3×10^{6}	96×10^{4}	2×10^{5}	8×10^{4}	*	*	*	<200	*	*
3	String beans.......	122×10^{4}	125×10^{3}	3×10^{4}	4×10^{3}	86×10^{4}	*	*	*	260	*	*
4	String beans.......	84×10^{4}	6×10^{6}	16×10^{4}	8×10^{3}	5×10^{4}	*	*	*	1220	*	*
5	String beans.......	1×10^{6}	144×10^{4}	16×10^{4}	7×10^{6}	3×10^{6}	104×10^{5}	2×10^{3}	3×10^{5}	1×10^{2}	$<10^{3}$	<10
1	Shrimp...............	3×10^{6}	323×10^{6}	8×10^{6}	36×10^{6}	22×10^{4}	19×10^{6}	7×10^{5}	$<10^{4}$	4×10^{4}	127×10^{3}	3×10^{5}
2	Shrimp...............	1×10^{5}	26×10^{4}	16×10^{4}	277×10^{3}	2×10^{5}	131×10^{4}	398×10^{4}	103×10^{4}	58×10^{4}	6×10^{5}	97×10^{4}
3	Shrimp...............	3×10^{6}	8×10^{7}	162×10^{5}	7×10^{6}	72×10^{7}	144×10^{5}	81×10^{4}	3×10^{5}	227×10^{4}	109×10^{4}	151×10^{4}
4	Shrimp...............	9	$>10^{4}$	$>10^{4}$	17×10^{7}	462×10^{6}	25×10^{6}	232×10^{5}	14×10^{7}	33×10^{4}	24×10^{6}	5×10^{7}
1	Salmon...............	18×10^{5}	35×10^{7}	1×10^{7}	*	*	57×10^{4}	*	15×10^{6}	3×10^{5}	*	*
2	Salmon...............	73×10^{2}	28×10^{6}	6×10^{6}	9×10^{6}	1×10^{7}	*	*	*	*	28×10^{4}	$<10^{4}$
3	Salmon...............	8×10^{5}	83×10^{4}	186×10^{5}	22×10^{5}	12×10^{5}	96×10^{5}	25×10^{5}	31×10^{5}	54×10^{3}	143×10^{5}	2×10^{3}
4	Salmon...............	7×10^{5}	67×10^{5}	9×10^{6}	162×10^{4}	22×10^{5}	174×10^{5}	78×10^{4}	48×10^{5}	64×10^{4}	$<10^{4}$	11×10^{4}
1	Peaches..............	51×10^{4}	40	0	1	1	*	*	*	*	0	1
2	Peaches..............	217×10^{4}	1	2	1	0	*	*	Con.**	*	*	*
3	Peaches..............	156×10^{4}	63	1½***	½***	½***	*	*	½***	½***	½***	0
4	Peaches..............	73×10^{4}	40	2	0	1	*	*	0	0	½***	Con.**
5	Peaches..............	109×10^{4}	36	1	0	0	*	*	2	2	Con.**	*
1	Tomato juice......	1×10^{6}	46×10^{4}	$<10^{3}$	26×10^{2}	<10	36	2	0	0	0	0
2	Tomato juice......	8×10^{5}	64×10^{5}	54×10^{3}	204×10^{3}	140	73×10^{2}	0	45×10^{2}	0	3×10^{2}	0
1	Peas....................	....	168×10^{7}	74×10^{6}	204×10^{7}	48×10^{4}	*	*	24×10^{7}	8×10^{3}	*	Con.**
2	Peas....................	....	17×10^{7}	8×10^{7}	109×10^{7}	72×10^{3}	*	*	122×10^{6}	*	5×10^{6}	Con.**
3	Peas....................	....	44×10^{6}	64×10^{7}	42×10^{6}	58×10^{3}	*	*	256×10^{3}	3	23×10^{5}	Con.**
4	Peas....................	....	17×10^{7}	8×10^{5}	*	*	2×10^{4}	*	1×10^{8}	*	*	*
5	Peas....................	....	111×10^{7}	4×10^{8}	25×10^{8}	2×10^{5}	*	*	*	*	$<2,000$	$<10^{4}$
1	Corn....................	25×10^{4}	16×10^{7}	2×10^{7}	8×10^{8}	252×10^{4}	*	*	*	*	1,080	2
2	Corn....................	107×10^{4}	3×10^{10}	224×10^{4}	132×10^{7}	4×10^{5}	*	*	226×10^{5}	*	*	45
3	Corn....................	77×10^{4}	2×10^{9}	368×10^{5}	18×10^{7}	4×10^{5}	*	*	63×10^{5}	33	37×10^{5}	Con.**
4	Corn....................	36×10^{4}	188×10^{7}	215×10^{6}	8×10^{8}	584×10^{8}	*	*	104×10^{4}	82	5×10^{6}	*

* No sample taken. ** Contaminated. ***Average count on duplicate plates.

The total number of organisms inoculated per can of food was determined by plate counts. The number of organisms inoculated per milliliter of fluid contents was calculated by dividing the total inoculum by the total number of milliliters in the capacity of the can.

Duplicate cans were always inoculated; one was incubated at 22°C. (71.6°F.) and the other at 37°C.(98.6°F.).

At different time intervals the cans were removed from the incubators, shaken, and samples taken. Decimal dilutions were made in sterile .85 per cent salt solution. Pour plates were made with one milliliter of the various dilutions in veal infusion agar. After 48 hours of incubation at 37°C., the colonies were counted.

Each test with the various items of food was repeated in some cases as many as five times. Counts of each sample made after 2, 7, 14, 30, and 60 days' incubation are shown (Table 1).

In canned corn and peas gas developed, causing the ends of the cans to bulge (swell). Some swelling of the cans occurred at 22°C. and more

TABLE 2

pH Values of Various Canned Foods Inoculated With a Food-Poisoning Strain of Staphylococcus aureus and Incubated for One Month at Temperatures of 22 and 37°C.(71.6 and 98.6°F.)

Food	22°C.	37°C.	Control (uninoculated)
Asparagus	4.37	4.35	5.49
Spinach	5.21	5.22	5.37
Salmon	6.05	5.97	6.23
Shrimp	6.50	6.89	6.61
Peaches	3.70	3.95	3.87
Tomato juice	4.17	4.20	4.15
Peas	5.05	4.83	6.00
Corn	......	4.62	6.39

at 37°C. after 48 hours' incubation. More gas was produced in canned peas than in canned corn. An analysis was made of the gas produced in peas and was found to be composed of CO_2—64.1 per cent, O_2—3.7 per cent, H_2—.6 per cent, saturated hydrocarbons—.3 per cent, and N—31.3 per cent.[2]

Hydrogen-ion determinations were made using a glass electrode potentiometer on duplicate cans of food after inoculation and incubation for one month at 22 and at 37°C. (Table 2).

DISCUSSION

The strain of *Staphylococcus aureus* grew in most of the foods tested (Table 1). Growth did not occur in tomato juice and peaches. In string beans, asparagus, and spinach there was less growth than in peas, corn, and the fish products.

The foods with highest acid content are tomato juice and peaches; asparagus, string beans, and spinach are semiacid; and peas, corn, shrimp,

[2] We are indebted to Mr. W. R. Homan of the Commonwealth Edison Company for the gasometric analysis.

and salmon are of low-acid content. There appears to be a direct correlation between pH and growth of staphylococci.

One very striking feature is that more growth was obtained at 22 than at 37°C. In only a few isolated instances was the bacterial count higher in the cans incubated at 37°C. (Table 1).

One of the difficulties encountered in the sampling of canned foods is the solid nature of the material. In these experiments, for practical purposes, only the fluid portion was sampled; hence the results may be different from those obtained if a sample containing both solid and liquid is used. However, before samples were taken the cans were thoroughly shaken.

Variation in counts on the same product may be due to differences in the chemical composition of the product. Different batches of the same canned product may vary as a medium for bacteria. These differences may be influenced by packing at different stages of maturity, the variety of vegetables canned, and seasonal conditions affecting the growth of the plant. All of these factors may contribute to changes in the chemical composition of the plant at the time of packing. No attempt was made to use the same lot number of samples and the cans were purchased from one local store as needed. Cans of the same item of food, therefore, may have been purchased at different times.

Growth of staphylococci in canned foods is not necessarily correlated with enterotoxin production. A striking example of failure to produce enterotoxin where good growth occurred is the case of canned salmon. In canned corn and oysters, however, enterotoxin was associated by Davison and Dack (1942) with the growth of staphylococci. Nevertheless, one can conclude that in the absence of growth of staphylococci no enterotoxin is produced, Segalove and Dack (1941).

In view of the fact that staphylococci are known to ferment the common laboratory sugars with only acid production it is difficult to explain the formation of gas in canned peas and corn.

SUMMARY

Canned foods of low-acid content (peas, corn); semiacid content (asparagus, spinach, string beans); acid content (tomato juice, peaches); and canned fish (salmon, shrimp) were experimentally inoculated with a food-poisoning strain of *Staphylococcus aureus*.

Growth was found to be best in the low-acid foods but was not found to be affected by the kind of food. In high-acid foods growth did not occur.

In almost all cases growth was better at 22 than at 37°C.

The staphylococcus produces acid but no gas in the carbohydrates which it ferments; however, in canned peas and corn gas is produced. More gas is produced in peas than in corn and the chemical composition of the gas is given.

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THIAMIN CONTENT OF FRESH AND FROZEN VEGETABLES [1,2]

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(Received for publication, July 20, 1942)

The preservation of vegetables by freezing is a relatively new industry and it is becoming more important year by year. With this new industry have come questions regarding the nutritive value of frozen vegetables. The quantity of vitamin B_1 present in the American diet is a source of concern since there are not many foodstuffs that are a potent source of the vitamin. Therefore, while vitamin B_1 is fairly widely distributed, it is necessary to have an understanding of the losses of it inherent in the processing of foodstuffs.

If the losses at various stages in processing are to be recorded, a method more rapid than the conventional bioassay is required. In an earlier paper by Moyer and Tressler (1942) a modification of the Hennessy-Cerecedo thiochrome procedure and sulfite cleavage in the fermentation test have been compared with the bioassay values in the determination of thiamin in eight frozen vegetables. It was found that each of these procedures gave results which were substantially in agreement with the others .

Previous studies on the losses of thiamin during processing prior to freezing have all been made with bioassay technique. The bioassay of necessity must be carried out over a period of time, during which the supplements, such as fresh or partially processed vegetables, would deteriorate and might give unreliable values. Therefore, the thiochrome method, which is much shorter, was used in these studies.

Rose and Phipard (1937) determined the vitamin B_1 content of peas in the fresh state and when frozen and found no loss. Fellers, Esselen, and Fitzgerald (1940) reported that little thiamin was lost during the preparation for freezing of peas and spinach, but that lima beans and asparagus lost 54 and 26 per cent, respectively. This decrease in the vitamin content was attributed to a longer blanching period for the latter vegetables. Fincke (1939) blanched peas for various temperatures and times, and reported that those blanched at 71°C.(159.8°F.) for two minutes showed the highest values and that those scalded for longer times and at higher temperatures tended towards lower thiamin values. She also noted no significant difference in thiamin content between peas blanched in steam and in hot water at the same temperature and for the same period of time. No appreciable loss of thiamin was noted in samples held at room temperature for four to eight hours prior to freezing. Farrell (1940) found that blanched frozen beans contained nearly 20 per cent more thiamin than

[1] Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 518, July 16, 1942.

[2] A résumé of a part of the thesis submitted by James C. Moyer to the Graduate School of Cornell University in partial fulfillment of requirements for the degree of Doctor of Philosophy.

the fresh vegetable. This increase in vitamin potency in the blanched product was attributed to the absence of active enzymes and increased digestibility.

EXPERIMENTAL PROCEDURE

Of eight vegetables commonly grown in this region, peas and asparagus were found in this laboratory to have thiamin contents sufficient to make a significant contribution to the daily diet, Moyer and Tressler (1942). Consequently the thiamin content of these two vegetables was studied during processing prior to freezing and after subsequent cold storage.

TABLE 1

Thiamin Content of Asparagus at Various Stages in Processing Prior to and After Freezing

Vegetable sample	Harvested May 12, 1941			Harvested June 12, 1941		
	Total solids	Micrograms thiamin per gram		Total solids	Micrograms thiamin per gram	
		Fresh wt.	Dry wt.		Fresh wt.	Dry wt.
	pct.			*pct.*		
Raw	9.34	1.83	19.6	8.40	1.74	20.7
Washed and cut	9.10	1.80	19.8	7.86	1.84	23.4
After blanching	9.15	1.60	17.5	7.73	1.56	20.2
After cooling	8.93	1.53	17.1	7.63	1.32	17.3
After freezing	8.70	1.52	17.5	8.51	1.46	17.2

For these studies samples taken from one load or field were kept separate and processed according to regular commercial procedure. Two separate studies were made on the losses in processing, one at the beginning of the season and the other at the end. As each lot of vegetable passed through the plant, samples were taken after each operation, ground to a paste, and portions taken for moisture and thiamin determinations; the results are presented (Tables 1 and 2).

TABLE 2

Thiamin Content of Peas at Various Stages in Processing Prior to and After Freezing

Vegetable sample	Harvested June 20, 1941			Harvested July 16, 1941		
	Total solids	Micrograms thiamin per gram		Total solids	Micrograms thiamin per gram	
		Fresh wt.	Dry wt.		Fresh wt.	Dry wt.
	pct.			*pct.*		
Freshly harvested	16.97	3.11	18.32	18.62	4.33	23.28
Before blanching	16.33	3.08	18.85	18.82	4.21	22.36
After blanching	16.78	2.92	17.93	17.33	3.61	20.82
After quality separation	16.17	2.87	17.74	18.23	3.42	18.75
Before packaging	16.08	2.84	17.65	16.95	2.95	17.40
After freezing	15.69	2.83	18.03	18.75	3.36	17.92

There appears to be a slightly higher thiamin content in the "washed and cut" samples than in the "raw." This may be due to error in sampling since the "washed and cut" material was carefully graded according to

size of stalk. If the "washed and cut" is taken as the criteria, then the amount of thiamin lost during processing would be 16 and 20 per cent, respectively.

When the first lot of peas was processed, there was a delay of 16 hours between the time of harvesting in the field and vining. During that interval the vines were spread out in a thin layer over the viner-shed floor. There was another delay of three hours between the time of shelling and washing. In spite of these delays there was no loss of thiamin according to the values shown (Table 2). The loss of thiamin during the processing of the first sample of peas was small, being about five per cent. The second lot of pea samples showed a much greater loss, about 25 per cent. This increased loss in the second study may be due to the difference in the variety, the first being Thomas Laxton and the second Telephone, or to differences in the peas because of changed climatic conditions. The greatest losses of thiamin occurred after blanching and quality separation.

TABLE 3

Conservation of Thiamin in Frozen Asparagus at Low Temperatures

Storage temperature	Time in storage	Total solids	Micrograms thiamin per gram	
			Fresh wt.	Dry wt.
	mo.	*pct.*		
	0	8.32	1.58	19.0
—12°C.(10°F.)	7	8.69	1.68	19.4
—22°C.(—8°F.)	7	8.60	1.57	18.2
—40°C.(—40°F.)	7	7.93	1.52	19.2

TABLE 4

Conservation of Thiamin in Frozen Peas at Low Temperatures

Storage temperature	Time in storage	Total solids	Micrograms thiamin per gram	
			Fresh wt.	Dry wt.
	mo.	*pct.*		
	0	17.97	3.68	20.5
—12°C.(10°F.)	5	17.63	3.59	20.4
—22°C.(—8°F.)	5	17.43	3.62	20.8
—40°C.(—40°F.)	5	17.98	3.68	20.4

CONSERVATION OF VITAMIN B_1 DURING STORAGE

There is a paucity of information concerning the loss of thiamin from frozen vegetables during storage. To obtain this information a quantity of commercially frozen asparagus and peas was stored for seven and five months, respectively, at —12, —22, and —40°C. (10, —8, and —40°F.). Analyses for thiamin and moisture were conducted on the ground vegetable after it had passed through a food chopper at —22°C. The thiamin and moisture analyses are presented (Tables 3 and 4).

Thiamin is not readily oxidized. Therefore, it is unlikely that it would be lost from a vegetable stored at a low temperature. The data presented in Table 3 verifies this conclusion.

SUMMARY

The thiamin content of asparagus and peas was determined by the thiochrome technique when the vegetables were freshly harvested, at various stages in the processing, and after freezing. These studies were conducted at the beginning and end of the processing season. The losses during processing varied from 16 to 20 per cent for asparagus and from five to 25 per cent in the case of peas. The greater loss in each case took place at the end of the season for the particular vegetable. Delays in the processing of one lot of peas showed that this vegetable could be held for some time at room temperature without any destruction of thiamin.

Samples of asparagus and peas were stored for seven and five months at —12, —22, and —40°C. (10, —8, and —40°F.). No significant loss of thiamin occurred in these vegetables at any of the storage temperatures.

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A NEW METHOD FOR STUDYING THE EFFECT OF BACTERIA ON BUTTER FLAVOR

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To bacteriologists, the solution of problems concerned with food spoilage depends largely upon one major criterion—namely, whether or not the abnormality dealt with can be produced at will by the use of certain cultures. Frequently the application of this criterion is difficult to arrange for, and may be one of the barriers to a more fundamental understanding of food spoilage. There are two main reasons for this difficulty; they are the complexities involved in duplicating (1) the product materials (especially on a small scale) and (2) the process of manufacture.

The first of these varies with both the product and its defect; the second, however, presents an opportunity for possible standardization. In the case of butter, this is to be dealt with.

"Sweet cream butter" as made exclusively in Canada and extensively in the United States, Australia, and New Zealand, is quite uniformly influenced by cream grading, neutralization of cream acidity, pasteurization, and the mechanical equipment used in its manufacture. According to Shutt (1929), Derby and Hammer (1931), Claydon and Hammer (1939), Linneboe (1940), and Jamieson (1941) microbial agencies likely to cause defects in the flavor of such butter originate generally from impure water or unsanitary equipment, notably churns. They become incorporated at some phase of processing subsequent to pasteurization.

Therefore in attempting to establish bacteria as the causative agencies of flavor defects it seems logical that those in question be inoculated directly into experimental lots of butter and there dispersed sufficiently to favor their activity.

Securing this effective association between bacteria and the product ordinarily requires considerable time, labor, material, and equipment, most of which are often difficult for the bacteriologist to provide, especially if he is located at considerable distance from a creamery. In such circumstances the assignment must be given over to the expert in dairy manufacture, or in some manner accomplished by the bacteriologist.

The method herewith described has proved sufficiently simple, reliable, and effective to seem worthy of being made known to other investigators.

EXPERIMENTAL METHOD

The technique may be termed the "butter-shake method." It was instituted primarily for testing the effect of pure cultures on butter, but later was found advantageous in qualifying the suitability of waters for creamery use. Therefore it has possibilities of moderately wide application.

Butter of clean flavor and low microbial content is melted to a creamy consistency and poured aseptically in 20-ml. amounts into special, wide-

mouthed, screw-capped, glass vials, four inches high and one inch in diameter, giving a 45-ml. capacity. These vials of butter are refrigerated until required.

To secure dependable butter, it has been found advisable to prepare it specially. The required small amount is easily made in any bacteriology laboratory. Difficulties of incorporating salt and moisture, as usually experienced in working small churnings, are avoided by melting and shaking the mass of butter before it is run into the sterile vials. Thus the bacteriologist can have a "butter medium" in the form sizes, and numbers most applicable to his needs.

INOCULATION AND DISPERSION OF TEST ORGANISMS

Inoculation from desired concentrations of individual pure cultures, combinations, or naturally occurring mixed cultures, such as in creamery waters, is made with ease and uniformity by the use of pipettes, the butter medium being retained in solid form until transfers are complete. Then the inoculated butters are melted to a mixable consistency.

Thorough dispersion of the inoculum is obtained by vigorous agitation for 20 minutes in a mechanical shaker. At average room temperature this shaking can be accomplished while the butter still is mixable, after which it is solidified quickly by cooling. The butter cultures, with the vial caps loosened one-half turn, are then incubated at the desired temperatures and for the time most favorable to the development of detectable flavors, usually six to eight days at 18°C.(64.4°F.).

FLAVOR DETERMINATION

The only innovation from usual procedure in detecting abnormalities of flavor is the use of suitable spatulas or triers for withdrawing from the vials sufficient butter to taste. Strips of 20-gauge stainless steel, five inches by five-eighths inch, and preferably shaped longitudinally to a half-round, are effective for this purpose. They are reasonable in cost, simply made, and durable.

EXPERIMENTAL VERIFICATION OF METHOD

The reliability of the "butter-shake method" is based upon the agreement between the results obtained with it and those from what might be termed the "individual working method." The latter closely simulates the procedure generally used in experimental work, as referred to in researches by Derby and Hammer (1931), White (1940), Long and Hammer (1941), Collins and Hammer (1933), and others, whether the inoculum was added preparatory to churning or at the washing or working stages. This seems a satisfactory criterion despite the appreciation that neither method produces the dispersion secured in commercial butter manufacture by mechanical equipment.

The inoculum, duplicating that tested by the "butter-shake method," was added to sterile water or naturally contained in the samples of creamery water being tested. These waters were used to wash 50-gram lots of butter granules weighed into four-ounce sterile jars after being aseptically churned from highly pasteurized cream. Considerable of the third wash water and .75 per cent of sterile salt was incorporated into each of the test butters

TABLE 1

A Comparison of Two Methods of Incorporating Test Inoculum Into Experimental Butter

Test cultures		Flavor scores and descriptions of inoculated butters after incubation at 18°C.(64.4°F.) for 12 days			
		Incorporation by hand workers		Incorporation by butter-shaking	
No.	Description	Score	Description	Score	Description
1	*Pseudomonas aeruginosa*	38.5	Slightly unclean	39	Reasonably clean
2	*Pseudomonas fluorescens*	37	Slightly rancid	36	Rancid
3	*Pseudomonas achromobacter* (Iowa)	36	Moderately putrid	34	Surface taint, cheesy
4	*Pseudomonas achromobacter* (O.A.C.)	36.5	Slight surface taint	36—	Surface taint
5	*Pseudomonas achromobacter* (U. of M.)	36	Surface taint	35	Strong surface taint
6	*Pseudomonas mucidolens* (Iowa)	35	Decaying potatoes	34	Strong decaying potatoes
7	*Pseudomonas mucidolens* (O.A.C.)	35.5	Decaying potatoes	34.5	Moderate decaying potatoes
8	Achromobacter sp. (?)	37	Decaying vegetables	35	Strong decaying cabbage
9	Pseudomonas sp. (?)	36	Rancid	35	Butyric
10	Combination of 2 & 3	36	Rancid	35	Rancid, bitter
11	Combination of 2 & 6	35	Potato decay	34—	Strongly musty
12	Combination of 2, 3, & 6	35—	Very unclean, objectionable	33	Too bad to describe
13	Creamery well water	38	Slightly unclean	37	Unclean
14	Creamery well water containing fluorescent bacteria	37	Unclean	36	Slightly rancid, cheesy
15	Churn rinse water (containing *Pseudomonas putrefaciens*)	37	Unclean	36—	Surface taint to slightly putrid
16	Good creamery well water	40	Clean	40	Clean
17	Controls	40	Clean	40	Clean

by means of sterile, wooden, hand workers, provided with shields for protection against contamination. The finished experimental butters were incubated in these same jars with the screw caps loosened one-half turn.

The results (Table 1) indicate that the method is equal to, or slightly more sensitive than, the individual working method in proving bacteria capable of causing deterioration in the flavor of butter, at least when they are introduced at the washing or working phase of butter manufacture.

Advantages of Butter-Shake Method: The method possesses the following advantages over those generally employed:

1. Economy of
   (a) Materials (cream and butter); from one gallon of cream testing 30 per cent fat, sufficient butter may be prepared for 65 individual tests.
   (b) Labor; labor is saved throughout inoculations, dispersion, and general handling. The feature of being able to store the test butter, in readiness for emergency requirements, permits of allocating the laborious phases to the most opportune times. Most of the labor becomes bacteriological technique.

2. The method of preparing and subdividing the required small initial lot of butter favors uniformity of product and extensive replication.

3. The product is packaged in a form favorable to prolonged preservation, ready for use and easy handling.

4. Owing to its method of preparation, the product permits of controlled variations in its natural or acquired constituents, any of which may have a critical bearing upon the detrimental activities of the test bacteria.

5. The concentration of the inoculum can be accurately controlled.

6. Combinations of pure cultures can be accurately effected and tested.

7. Water supplies detrimental to the flavor of butter can be detected. The small amount of inoculum required for each vial of butter avoids the necessity of obtaining the usual large-sized samples.

8. Extensive replication of inoculum, variations of constituents, different temperatures of incubation or storage, and other influential factors can be tested with a minimum of effort.

9. Dispersion of the inoculum is quite equal in uniformity to that obtained by usual methods of hand-operated workers.

SUMMARY

Technique termed the "butter-shake" method is described and suggested as a most advantageous and efficient means for proving bacteria to be responsible for defective flavors in butter.

The method brings this essential phase in the analysis of flavor defects under complete bacteriological control. It provides a "butter medium" favorable to extended preservation, ready use, ease of handling, controllable variation, extensive replication, and is applicable to tests from pure cultures, arranged combinations, or those in direct inoculations from creamery waters. The details of these and other advantages are presented.

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MICROBIAL DESTRUCTION IN BUFFERED WATER AND IN BUFFERED SUGAR SIRUPS STORED AT —17.8°C.(0°F.)[1]

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The experimental studies which follow are concerned with the comparison of buffered water and buffered 30-per cent sucrose, dextrose, and invert sirups, with respect to their relative influences on microbial destruction at —17.8°C.(0°F.). The chemical and physical differences between the sugars naturally lead to the supposition that microbial forms suspended in highly concentrated solutions might be destroyed with greater rapidity in a frozen solution of one kind of sugar than in a frozen solution of another kind. Interest in the comparative food-preservation values of these three sugars is not new. It arises again because new processing methods enable the manufacture of purer grades of dextrose and invert sugars than have hitherto been possible and because there is a desire to substitute one or the other of these sugars for sucrose, wholly or in part, whenever such a substitution would be advantageous either for improvement of the product or for economic reasons.

Although sucrose has long been used as a preservative in many foodstuffs, not too much thought has been given to the possibility that it might retard or even inhibit microbial destruction in the foodstuffs which it is preserving. A philosophical thankfulness for the good things we have has arrested much of our curiosity as to the extent of actual microbial destruction in sugar-preserved foods and as to whether one kind of sugar is a better preservative than another, at least from a microbiological standpoint. In recent years fundamental research on the influence of sugar on microbial behavior has been stimulated (1) by observations that some microorganisms not only remain alive in pure sugar, but that they may bring about spoilage in products containing high concentrations of sugar; namely, in fruit concentrates, dry sugar-packed and sirup-packed frozen fruits, preserves, molasses, sorghum sirup, corn, maple and purified cane and beet sugar sirups, honey and candies; and (2) by a desire to enhance existing knowledge of the preservation in concentrated sugar solutions of pure (stock) cultures of microorganisms.

REVIEW OF LITERATURE

For many years plant physiologists have been aware that high concentrations of sugar in solution protect plant cells against destruction by temperatures below freezing. Chandler and Hildreth (1936) discuss some of the conclusions reached by earlier investigators regarding this phenomenon and present their own. As recorded in the tabular data presented by these workers, greater destruction of Lukens Honey peach pollen occurred in a saturated dextrose solution than in a saturated sucrose solution stored at

[1] Agricultural Chemical Research Division Contribution No. 77.

—15 to —17°C. (5 to 1.4°F.). However, the soluble-solid content differs in the two saturated solutions. Keith (1913) kept agar slant cultures of bacteria in a vigorous condition for eight months by covering the growths with sterile 10 per cent cane sugar (sucrose) solution and by storing at —10°C.(14°F.). Vass (1919) suspended *Bacillus radicicola* in batches of a nutrient medium containing .01 to 15 per cent dextrose, and froze samples of the suspensions at —15 and —190°C. (5 and —310°F.). According to his results the concentration of the medium had no effect when the temperature was lowered below the eutectic point of the sugar, but at temperatures above the eutectic point the sugar showed a marked protective action. Tanner and Wallace (1931) reported that suspensions of microorganisms frozen in distilled water and in various concentrations of sugar sirup showed very slow decrease in viable cells. McFarlane (1940, 1941, 1942) found that high concentrations of sucrose (30, 40, and 50 per cent) tended to greatly delay destruction of microorganisms at subfreezing temperatures and that the extent of destruction was influenced by such factors as the nature of the microorganism, storage temperature, hydrogen-ion concentration, total acidity of the medium, storage interval, etc.

The greater part of the literature, concerning the influences of the sugar concentration-temperature relationships on microbial destruction, is concerned with the influences exhibited at temperatures above freezing. Sucrose and dextrose have received the most attention. Owen (1926) found *Torulae* more resistant to heat when exposed in the higher density sirups and more resistant in corn sirup than in cane sirup. Rahn (1928) added different concentrations of cane sugar to a raisin extract and observed that at a temperature from 127 to 132°F. (52.8 to 55.6°C.) the higher concentrations of sugar offered greater protection to suspended yeast cells. Buchanan (1932) comparing refined corn sugar (dextrose) with sucrose solutions, found that at higher temperatures yeast spores were killed more easily in sucrose solutions, whether or not small amounts of citric acid were added, but that at lower temperatures dextrose (corn sugar) solutions had a greater inhibiting action to the growth of yeast spores. Fay (1934) noted that all cells did not exhibit the phenomenon of increased thermal resistance in hypertonic solutions and that equimolal solutions of different sugars did not show the same protective action. He wrote that "when certain cells are heated in a series of solutions of dextrose with increasing osmotic pressures, there is an unmistakable parallel increase in the protective action. A similar series of equimolal concentrations of sucrose will show considerably greater protective action than the dextrose solutions. . . . Maltose and lactose gave little or no protection to the cells studied". Baumgartner and Wallace (1934) likewise observed that the thermal resistance of some microorganisms increased with increasing concentrations of sucrose. They found little difference in the protective action exerted by buffered 20 per cent sucrose, dextrose, and invert sugar solutions (pH 7) at 60°C.(140°F.).

In some of Morrison's (1938) experiments greater reductions in the number of microorganisms occurred in the dextrose sirups than in the sucrose sirups of corresponding concentrations, but no marked differences were observed. Nunheimer and Fabian (1940), studying the influence of

organic acids, sugars, and sodium chloride upon strains of food-poisoning staphylococci at room temperature, found that "Dextrose exerted an inhibitive effect in concentrations of 30 to 40 per cent and a germicidal effect at 40 to 60 per cent." Also, that "Sucrose is less active than either dextrose or sodium chloride since a concentration of 50 to 60 per cent was required for inhibition and 60 to 70 per cent for germicidal action." In "a mixture of sugars and acid, it was found that dextrose was more effective than sucrose in lower concentrations of acids." A study similar to the above was made by Erickson and Fabian (1941) on yeasts and similar results were obtained insofar as the relative influences of sucrose and dextrose were concerned.

EXPERIMENTAL PROCEDURE

The microorganisms used in the following experiments, their plating media, incubation periods, and history are listed (Table 1). All agar slant

TABLE 1

Microorganisms Used in Experiments, Their Plating Media, Incubation Periods, and History

Microorganism	Plating medium	Incubation period	History, source, etc.
	agar	*days*	
Escherichia coli	Dextrose [1]	3	Laboratory stock culture
Torula species (pink)	Dextrose	4-5	Frozen orange juice
Aspergillus nidulans (spores)	Wort [2]	2-3	Canning grade cane sugar
Sacoharomyces cerevisiae	Wort	3	Hall, James, and Stuart (1933)
S. ellipsoideus Hansen	Wort	3	Champagne variety, original culture from Baarn, Holland
Brewers' yeast	Wort	4-5	Local brewery
Schizosaccharomyces octosporus	Wort	4-5	Department of Bacteriology, Michigan State College, East Lansing
Zygosaccharomyces pastori	Wort	4-5	Department of Bacteriology, Michigan State College, East Lansing
Z. japonicus	Wort	4-5	Department of Bacteriology, Michigan State College, East Lansing
Unidentified yeast	Wort	3	Frozen sirup-packed Boysenberries—stored over three years at —17.8°C.

[1] Dextrose agar = nutrient agar + one per cent dextrose. pH range of different batches from 6.6 to 7.1 [2] Wort agar = 100 grams malt sirup + 15 grams agar + 1,000 ml. distilled water, pH range = 4.9 to 5

cultures and plates were incubated at 30 C.(86°F.). Suspensions were prepared for freezing from three-day-old nutrient agar slant cultures of *Escherichia coli;* four-day-old nutrient agar slant cultures of the Torula species; 13-day-old wort agar slant cultures of *Aspergillus nidulans;* and three- to four-day-old wort agar slant cultures of the yeasts. The growths were rinsed from the slants with small amounts of sterile distilled water and the resulting heavy suspensions shaken with glass beads. Measured amounts of these suspensions were used for inoculating the buffered media.

The media, distilled water and 30 per cent sucrose, dextrose, and invert sirups, were buffered with McIlvaine's (1921) disodium phosphate-citric

TABLE 2

Microbial Destruction in Buffered Water and in Buffered Sugar Sirups Stored at —17.8°C.(0°F.)

Experiment	Medium	Plate counts							
		Before freezing	After freezing						
			24 hrs.	1 week					
Exp. 1—*E. coli*	W	30,300	0	0	....	....	....	....	....
	S	65,300	7,500	733	....	....	....	....	....
	D	67,300	2,283	7	....	....	....	....	....
	I	60,300	2,300	7	....	....	....	....	....
			24 hrs.	1 week	8 wks.	16 wks.	32 wks.	44 wks.	60 wks.
Exp. 2—Torula species (pink)	W	13,900	2,600	767	135	90	11	2	0
	S	18,900	14,100	18,300	15,150	13,100	5,400	2,683	255
	D	19,700	17,500	22,200	15,800	4,250	465	73	3
	I	19,400	18,100	19,600	16,400	12,233	6,900	7,300	2,722
			24 hrs.	1 week	8 wks.	16 wks.	32 wks.	44 wks.	60 wks.
Exp. 3—*A. nidulans* (spores)	W	30,000	15,100	18,200	11,600	8,100	3,000	1,700	580
	S	26,000	16,700	19,500	20,100	18,700	14,400	13,967	7,617
	D	17,500	14,700	19,300	24,300	24,433	10,700	2,833	367
	I	19,700	18,100	22,700	29,500	25,467	19,930	19,133	15,633
			24 hrs.	1 week	4 wks.	8 wks.	12 wks.	16 wks.	24 wks.
Exp. 4—*S. cerevisiae*	W	11,700	4,300	1,027	120	61	17	11	5
	S	11,250	10,050	7,544	4,550	3,050	2,200	2,193	1,200
	D	8,300	6,050	4,889	3,800	1,875	1,350	540	56
	I	9,500	7,650	6,189	3,734	2,225	1,075	540	149
			24 hrs.	1 week	4 wks.	8 wks.	12 wks.	16 wks.	28 wks.
Exp. 5—*S. ellipsoideus* Hansen	W	7,700	525	165	15	9	6	6	1
	S	5,600	3,250	2,550	1,163	595	310	245	127
	D	4,400	2,425	2,167	1,073	565	283	58	16
	I	4,500	2,925	2,300	853	330	236	116	38

TABLE 2 (Concluded)

			24 hrs.	1 week	4 wks.	8 wks.	12 wks.	16 wks.	28 wks.
Exp. 6—Brewers' yeast	W	12,500	7,050	2,984	217	32	2	1	0
	S	14,800	11,200	10,900	7,417	6,200	4,975	4,533	1,754
	D	13,500	11,900	10,000	8,400	4,950	1,275	258	1
	I	14,300	11,300	10,417	8,000	6,800	4,250	2,448	27
			24 hrs.	1 week	4 wks.	8 wks.	12 wks.	28 wks.	33 wks.
Exp. 7—*Sch. octosporus*	W	2,700	1,075	615	455	368	273	112	112
	S	2,600	2,500	2,325	1,515	1,155	853	690	677
	D	2.700	2,500	2,200	1,990	1,370	985	360	220
	I	2,500	2,625	2,525	1,975	1,000	700	460	342
			24 hrs.	1 week	4 wks.	8 wks.	13 wks.		
Exp. 8—*Z. pastori*	W	6,680	3,050	225	11	3	1	....	....
	S	6,140	6,050	3,375	2,325	720	79	....	....
	D	6,810	6,050	5,100	1,925	226	4	....	....
	I	7,000	6,150	4,025	3,225	1,580	503	....	....
			24 hrs.	1 week	4 wks.	8 wks.	13 wks.		
Exp. 9—*Z. japonicus*	W	1,900	650	25	1	0	0	....	....
	S	2,000	1,500	1,090	605	200	10	....	....
	D	1,900	1,450	1,085	260	29	2	....	....
	I	1,900	1,550	1,230	605	180	40	....	....
			24 hrs.	1 week	4 wks.	8 wks.	10 wks.		
Exp. 10—Unidentified yeast	W	3,300	1,250	50	2	2	1	....	....
	S	3,300	3,700	2,600	2,090	1,290	705	....	....
	D	3,100	3,200	2,675	613	34	2	....	....
	I	3,000	2,900	2,275	842	128	4	....	....
			24 hrs.	1 week	4 wks.	9 wks.	22 wks.	27 wks.	
Exp. 11—Unidentified yeast	W	275,000	82,000	9,300	550	65	38	7	....
	S	293,000	295,000	270,000	228,000	108,000	33,100	31,066	....
	D	312,000	283,000	225,000	85,000	1,300	4	3	....
	I	271,000	276,000	220,000	85,000	9,840	213	19	....

[1] W = water; S = 30 per cent sucrose sirup; D = 30 per cent dextrose sirup· I = 30 per cent invert sirup.

acid buffer of approximately half strength. In dispensed samples of the suspensions (in different experiments) the hydrogen-ion concentrations ranged from pH 3.2 to 3.3, and the titratable citric acid contents ranged from .74 to .77 gram per 100 milliliters; in the sirups the soluble-solid contents ranged from 29.8 to 30.4 per cent at 20°C.(68°F.). A sugar refractometer was used in making the sirups to the desired concentration. The sugars (a canning grade of cane sugar, dextrose hydrate, and a clear invert sirup) were commercial grades and possessed a high degree of purity. The buffered sirups were comparable in hydrogen-ion concentration, titratable acid, and soluble-solid content with the drained sirups from some frozen berries.

Flasks of media were cooled in a constant temperature room to 6°C. (42.8°F.) and inoculated at that temperature with the desired microorganism. Each thoroughly shaken suspension was removed to the laboratory and dispensed in 10-milliliter quantities in pyrex tubes (20 x 150 mm.). The tubes were stoppered with scalded corks and returned to the 6°C. room. Each suspension was exposed to room temperature for about nine minutes while dispensing. This exposure permitted the medium to warm up to about 14.6°C.(58.3°F.). These dispensing time and temperature figures are averages. It required six to 14 minutes to dispense a given suspension. The temperatures of the liquid in the dispensed tubes ranged, for different batches, from 11.5 to 17°C. (52.7 to 62.6°F.). Immediately after the four flasks of media utilized in each experiment had been dispensed, the racked tubes were transferred from 6°C. to —17.8°C. The time between the inoculation of the flasks at 6°C. and the storage of the tubes at —17.8°C. ranged from 40 to 50 minutes in the different experiments. Samples of all suspensions were plated at the time of storage to determine the viable cell content. Triplicate samples of the frozen *E. coli* suspensions were plated daily. Duplicate and, in many instances, triplicate samples of the other frozen microbial suspensions were plated on the first and fourth days and on the second, third, fourth, fifth, sixth, seventh, eighth, 10th, 12th, 14th, and 16th weeks and thereafter monthly, if necessary, to obtain further data. Frozen samples were thawed in cold water (5 to 10°C.) and plated as soon as thawed. The cells which failed to produce colonies are referred to as having been destroyed. It is realized, of course, that all the cells which fail to produce colonies may not be dead, McCulloch (1936).

EXPERIMENTAL RESULTS

Representative plate counts are given (Table 2), and the data include counts made on the microbial suspensions prior to freezing and counts made on samples which had been held for varying intervals of time at —17.8°C. (0°F.). The relationship of the buffered media in protecting the microorganisms against destruction during storage is shown (Table 3).

Escherichia coli, Experiment 1: The cells of this bacterial species were rapidly destroyed in all four media (Table 2). Greatest destruction occurred in water and least in the sucrose sirup, but even in this latter medium 98.9 per cent of the cells were destroyed by the end of the first week of storage at —17.8°C. Somewhat greater destruction occurred in the dex-

trose than in the invert sirup the first few days of storage, but by the end of the week over 99.9 per cent of the cells had been destroyed in both media. *E. coli* was rarely recovered from water suspensions which had been frozen 72 hours or longer, either in a dextrose agar culture medium or in lactose broth. The possibility was considered that the buffered suspending medium in the quantities used for the lower dilutions with the culture media may have lowered the hydrogen-ion concentrations of the mixtures sufficiently to inhibit growth. Therefore, pH determinations (glass electrode) were made on numerous samples of incubated culture media which had been inoculated with different quantities of the buffered water suspensions. The following results are typical:

TABLE 3

Relationship of Buffered Media in Protecting Microorganisms Against Destruction During Storage at —17.8°C.(0°F.)

Microorganism	Experiment No.	Increasing order	Relation apparent	
			From	*Through*
E. coli	1	W<D ? I<S[1]	1st day	1st week
Torula sp.	2	W<D<S<I	10th week	60th week
A. nidulans (spores)	3	W<D<S<I	36th week	60th week
S. cerevisiae	4	W<D ? I<S	20th week	24th week
S. ellipsoideus Hansen	5	W<D ? I<S	16th week	28th week
Brewers' yeast	6	W<D<I<S	10th week	28th week
Sch. octosporus	7	W<D<I<S	28th week	33rd week
Z. pastori	8	W<D<S<I	4th week	13th week
Z. japonicus	9	W<D<S<I	3rd week	13th week
Unidentified yeast	10	W<D ? I<S	3rd week	10th week
Unidentified yeast	11	W<D<I<S	1st week	22nd week

[1] W = water; S = 30 per cent sucrose sirup; D = 30 per cent dextrose sirup; I = 30 per cent invert sirup; ? = only a slight degree of difference, of doubtful significance.

	Lactose broth (10-12 ml.)	Dextrose agar (15-18 ml.)
1.0 ml. buffered water suspension	pH 4.28 to 5.08	pH 5.41 to 5.67
0.5 ml. buffered water suspension	pH 4.39 to 5.61	pH 5.98 to 6.17
0.1 ml. buffered water suspension	pH 6.00 to 6.82	pH 6.53 to 6.63

With one exception, one milliliter of buffered water suspension did not lower the pH of the lactose broth below pH 4.5 in any of the fermentation tubes examined. Although pH 4.5 is considered low, it was not inhibitive to the growth of *E. coli*. Lactose fermentation tubes (pH 4.5), failing to show growth after 48 to 72 hours at 37°C.(98.6°F.) when inoculated with one, one-half, or one-tenth milliliter portions of the buffered water suspensions, invariably showed growth and gas formation when reinoculated with cells from a known viable culture. This was expected since the investigations by Reid (1932) and by Nunheimer and Fabian (1940) have shown that citric acid exerts inhibitory or germicidal action only when present in relatively high concentration.

Torula Species (pink), Experiment 2: Greatest destruction of Torula cells occurred in water, but even in this medium a few cells survived over 44 weeks at —17.8°C. Plate counts made during the first eight weeks of storage did not show markedly greater destruction in one sirup than in

another, but counts made from the 10th week on were consistent in showing greater destruction in dextrose sirup than in sucrose and greater destruction in sucrose than in invert sirup. After 60 weeks at —17.8°C., 99.9 per cent of the cells had been destroyed in dextrose sirup, 98.7 per cent in sucrose, and 86 per cent in the invert sirup.

Aspergillus nidulans (spores), Experiment 3: Spores of this mold were very resistant. Large numbers remained viable for 60 weeks in all four media. Greatest destruction occurred in water and the least in the invert sirup. Greater destruction occurred in the dextrose sirup than in the sucrose. The media exhibited the same relationship to each other in influencing mold spore destruction that they exhibited in influencing the destruction of the Torula cells. The counts made on the dextrose and invert sirup suspensions before freezing were probably too low, since many counts made on stored samples of these suspensions were consistently higher. One milliliter of the buffered suspending medium lowered the pH of 15 milliliters of wort agar (pH 4.9) in the poured plates to pH 4.3; .5 milliliter, to pH 4.5; and .1 milliliter, to pH 4.8. These changes in pH did not seem inhibitory to the growth of mold or of the yeasts.

Saccharomyces cerevisiae and S. ellipsoideus Hansen, Experiments 4 and 5: These two yeasts responded in the same manner to their environments. Destruction was much greater in water than in the sirups. It was less in sucrose sirup than in either dextrose or invert sirups. Although the difference in the rate of destruction was not great in the dextrose and invert sirups, slightly greater destruction did occur in the dextrose sirup after several weeks' storage. After 24 weeks' storage, 99.9 per cent of the cells of *S. cerevisiae* were destroyed in water, 89.3 per cent in sucrose sirup, 99.3 per cent in dextrose sirup, and 98.4 per cent in invert sirup. After 28 weeks' storage, 99.9 per cent of the cells of *S. ellipsoideus* were destroyed in water, 97.7 per cent in sucrose sirup, 99.6 per cent in dextrose sirup, and 99.2 per cent in invert sirup.

Brewers' Yeast, Experiment 6: Samples of the frozen suspensions of brewers' yeast were plated over a 28-week period. At the termination of this period over 99.8 per cent of the cells in the water, dextrose, and invert sirups failed to give any indication of viability in wort agar. Approximately 88.2 per cent of the cells in the frozen sucrose sirup likewise failed to reproduce.

Schizosaccharomyces octosporus, Experiment 7: At the end of 33 weeks' storage, 95.9 per cent of the cells in the frozen water suspensions failed to produce colonies. Seventy-four per cent of the cells in the sucrose sirup, 91.9 per cent in the dextrose, and 86.3 per cent in the invert likewise did not reproduce. *Sch. octosporus* offered considerably more resistance to the less favorable environmental conditions than some of the other yeasts (Table 2).

Zygosaccharomyces pastori and Z. japonicus, Experiments 8 and 9: These yeasts responded to their environmental conditions in the same manner as the Torula species and the *A. nidulans* spores, i.e., the greatest destruction occurred in water (over 99 per cent the first week), least in invert sirup, and less in sucrose sirup than in dextrose. The experiments were

discontinued the 13th week because over 90 per cent of the cells failed to grow, and because there had been little change in the relative influences exerted by the media during the last nine to 10 weeks. By the 10th week over 99.5 per cent of the cells of both yeast species were destroyed in the dextrose sirup. By the 13th week, 98.7 per cent of the *Z. pastori* cells in the sucrose sirup, and 92.8 per cent in the invert were destroyed. In this same period 99.5 per cent of the *Z. japonicus* cells in the sucrose sirup and 97.9 per cent in the invert were destroyed.

Unidentified Yeast, Experiments 10 and 11: This yeast was selected because of the belief that it might be a variety particularly resistant to cold, since it had survived in a can of sirup-packed Boysenberries which had been stored for over three years at —17.8°C. The two experiments (10 and 11, Table 2) varied only in the number of viable cells originally suspended in the media. There was a rapid decrease in the number of viable cells in the frozen buffered water suspensions. After a few weeks' storage the total number of viable cells were much smaller in both dextrose and invert sirups than in the sucrose. The resistance of the organism to destruction in the buffered sucrose sirup was not surprising since the environmental conditions in this sirup were not essentially different from those in the frozen sirup-packed Boysenberries from which the organism was originally isolated.

DISCUSSION

Most of the *E. coli* cells had lost their viability in all four media by the end of the first week, whereas large numbers of *A. nidulans* spores remained viable for several months. Greater destruction of all the microorganisms investigated occurred in water than in the sirups. Prolonged storage at —17.8°C. brought about greater destruction of *S. cerevisiae, S. ellipsoideus* Hansen, *Sch. octosporus*, the brewers' yeast, and the unidentified yeast in dextrose and invert sirups than in sucrose. These same microorganisms were destroyed in greater numbers in dextrose sirup than in the invert, but the difference was not pronounced after several weeks' storage. Greater destruction of the Torula species, *A. nidulans* spores, *Z. pastori*, and *Z. japonicus* occurred in dextrose than in the other two sirups; and greater destruction occurred in the sucrose sirup than in the invert. It is interesting to note (Table 2) that although some microorganisms remain viable in greater numbers in sucrose and some in invert, none of the microorganisms investigated seem to fare equally well in the two sirups. It is also interesting to note (Table 3) that in some experiments the relative influences exerted by the different suspending media were observable after one to four weeks of storage and occasionally sooner, while in other experiments they were evident only after 10 to 36 weeks of storage. There are numerous experimental limitations and variables to be kept in mind in interpreting and applying the specific results obtained in any series of experiments. Prudden (1887) and Hilliard, Torossian, and Stone (1915) have called attention to many of them. Two particularly significant variables to be considered here are the following: first, the initial concentration of viable cells in suspension at the time of freezing (if only a few viable cells are in suspension, the greater portion of them may lose their viability in a very

short time, a few hours or a few days at the most, while many of the cells in a concentrated suspension may retain their viability several months or even longer); second, the storage intervals at which samples are plated and at which the relative values of the media in supporting microbial life are determined.

If suspending media differ in any phase of their composition, it is to be expected, other factors being equal, that the rates of approach to environmental equilibria will vary. Therefore, the relative values of different media in influencing microbial behavior cannot very well be determined until the maximum environmental changes have occurred for the particular experimental conditions. It is probably a fact that a true equilibrium is never attained under the usual experimental and commercial storage conditions because of the fluctuating temperature and the slowly changing crystalline structure. This, however, need not detract any from making practical use of the observation that certain microorganisms have less cold resistance in some kinds of sugar sirups than in others when the sirup suspensions are given the same treatment. From a microbiological standpoint, the experimental evidence indicates that there should be no discrimination against the use of dextrose or invert sirups in the preservation of frozen fruits and fruit products if these sugars are otherwise satisfactory. The observation that a high concentration of sugar tends to protect microorganisms against the rigors of their low-temperature environment confirms similar observations previously reported. Just why dextrose sirup offers less protection than sucrose or invert sirup is not clear. Differences in osmotic pressures of the sirups and in the crystalline structures of their masses may account for their respective influences on microbial destruction or survival at low temperatures. This explanation, however, needs more direct and more conclusive proof than has ever been given.

SUMMARY

Ten microorganisms, *E. coli*, a Torula species, *A. nidulans* (spores), *S. cerevisiae*, *S. ellipsoideus* Hansen, *Sch. octosporus*, *Z. pastori*, *Z. japonicus*, a strain of brewers' yeast, and an unidentified yeast, were each suspended in buffered water and in buffered 30 per cent sucrose, dextrose, and invert sirups and stored at —17.8°C. (0°F.). Different batches of suspending media varied in hydrogen-ion cencentrations from pH 3.2 to 3.3 and in titratable citric acid contents from .74 to .77 gram per 100 milliliters.

After prolonged storage at —17.8°C., which varied for the different microorganisms from one to 60 weeks, it was observed that:

1. Greater destruction of microbial cells occurred in water than in any of the sirups.

2. Greater destruction occurred in dextrose sirup than in either invert or sucrose sirup, but there was not always a marked difference in the degree of cold resistance evident in dextrose and invert sirups.

3. *E. coli*, *S. cerevisiae*, *S. ellipsoideus* Hansen, *Sch. octosporus*, the strain of brewers' yeast, and the unidentified yeast exhibited greater cold resistance in sucrose than in invert sirup. The Torula species, *A. nidulans* spores, *Z. pastori*, and *Z. japonicus* exhibited greater cold resistance in invert than in sucrose sirup.

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RELATION OF HYDROGEN-ION CONCENTRATION TO COLOR DEVELOPED IN CURED PORK [1, 2]

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The color of cured meat is generally recognized as an important factor in the determination of its market value even though it has not been demonstrated that the color actually affects the quality of the product.

Since the work of Haldane (1901), Hoaglund (1914), Günther (1921), and Urbain and Jensen (1940), it has been known that the red pigments of cured meat, nitric oxide myohemoglobin and nitric oxide hemoglobin, are formed by the myohemoglobin or hemoglobin respectively in the meat muscle, and the nitrite of the curing pickle. That the quality and uniformity of the color developed in the meat by this reaction is affected by other factors has been definitely established. The following list contains the known or suspected factors influencing the color development in cured meat:

1. Nitrite absorbed by the meat. (Enough nitrite must be present to form sufficient nitric oxide myohemoglobin to give a desirable color.)
2. Amount of myohemoglobin or hemoglobin in the tissue. (Enough myohemoglobin or hemoglobin must be present to produce a desirable color shade after the reaction with nitrite.)
3. Microorganisms. (Effect of nitrate reducing bacteria is important in nitrate cures in producing sufficient nitrite for the pigment reaction. Secondary effects of microorganisms in nitrite cures are rather obscure, but probably may enhance the reaction by helping to maintain reducing conditions or hinder reaction by oxidizing meat pigments.)
4. Sugars in the presence of microorganisms. (Sucrose is converted to reducing sugars by microorganisms which help maintain the reducing conditions in the meat necessary for normal nitric oxide myohemoglobin formation.)
5. Atmospheric oxygen. (May affect the color by oxidizing nitric oxide myohemoglobin to the brown pigment metmyohemoglobin.)
6. Ionic concentration of the pickle solution. (May speed up oxidation by lowering the pH.)
7. Oxidation-reduction potential of the meat. (Normally, the reducing conditions necessary for proper formation of nitric oxide myohemoglobin are present in the meat.)
8. Structure of the meat muscle. (Structure and arrangement of the muscle fibers may give slightly different optical properties and produce color differences.)
9. Temperature of curing. (Influence obscure; however, indirectly high temperature is an undesirable factor, since it favors accelerated bacterial growth.)
10. Desiccation. (Affects optical properties of the meat [reflection, etc.] and so affects color.)
11. pH. (Has effect on speed of nitrite-myohemoglobin reaction. Controversial evidence regarding effect on color.)

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[1] From a thesis submitted by P. C. Duisberg in partial fulfillment of the requirements for the Degree of Master of Science in Agricultural Biochemistry.

[2] Authorized for publication on June 13, 1942, as paper No. 1113 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

Among these factors, the data concerning the relationship between pH and color development are particularly scant and inconclusive. Moulton (1936) and Callow (1930) obtained indirect evidence of a relationship between the pH and the color of cured meat. Winkler, Hopkins, and Thistle (1940), using bacon sides, found a moderate degree of association between color quality and pH. Urbain and Jensen (1940), on the other hand, using buffered solutions of nitric oxide hemoglobin prepared from hogs' blood, found no significant spectrophotometric differences over a pH range of 5.1 to 8.

In view of the economic importance of obtaining desirable color formation in cured meats the present study was planned to obtain further data dealing with the effect of pH on color development in cured meat.

EXPERIMENTAL PROCEDURE

In order to study the relationship between the pH and the color developed in cured meats, it was necessary to develop a laboratory method of curing which would approximate actual curing conditions as closely as possible and in which the known variable factors in meat curing could be controlled. In order to minimize any variation owing to differences in the meat and the method of curing, a standard method of sampling and of curing was adopted.

Pork loin was cut into one-inch strips perpendicular to the rib bones. Fat deposits were trimmed away and one-inch cubes, weighing approximately 20 grams, were cut. Only samples of pork between pH 5.6 and 5.7 were used for curing. The pork samples were placed under a General Electric Germicidal Lamp for 10 minutes and then turned over with sterile forceps for an additional 10 minutes. At the same time, 25 milliliters of pickle solution were pipetted into a glass curing jar (height, three inches; diameter, one and one-half inches; having a ground glass stopper) and radiated under the lamp for 20 minutes. The pork was immersed in the pickle solution (with the forceps), the ground glass cover attached, and the samples stored in the refrigerator at 4°C.(39.2°F.).

After a curing period of 24 hours the moisture, salt, and nitrite contents were found to be within the limits found in cured pork and the color and flavor were characteristic of cured pork.

The pickle solution contained 24 per cent NaCl, nine per cent sucrose, .49 per cent $NaNO_3$, and .057 per cent $NaNO_2$, and had a salinometer value of 82° at 25°C.(77°F.). The pickle was made from C. P. salts dissolved in distilled water.

The growth of microorganisms was inhibited by placing the samples under the Germicidal lamp even though the work of Haines (1937) showed that the effect of microorganisms could not be great in curing periods of 24 hours at low temperatures. A subsequent study showed that in the described procedure there was no significant variation in the pH of the pork or the pickle after as much as seven days' storage.

Owing to the use of sucrose and the control of microorganisms, there was no positive test for reducing sugars with Fehling's solution until about the seventh day of curing. Therefore, the combined effect of microorganisms and sugars was controlled by the method of curing.

The pH measurements on the pork were made with a plunging glass electrode, and on the pickle with a glass electrode.

The moisture and nitrite contents were determined by the method of the A. O. A. C. (1935).

The NaCl was determined by the wet digestion method of Davies (1932).

Color was determined with a Bausch and Lomb reflectance spectrophotometer.

It was necessary to determine the normal changes in pH of the curing meat during the time of cure before studying any relationship of color. This study was extended for a period many times longer than the 24-hour cure. The results are illustrated (Fig. 1).

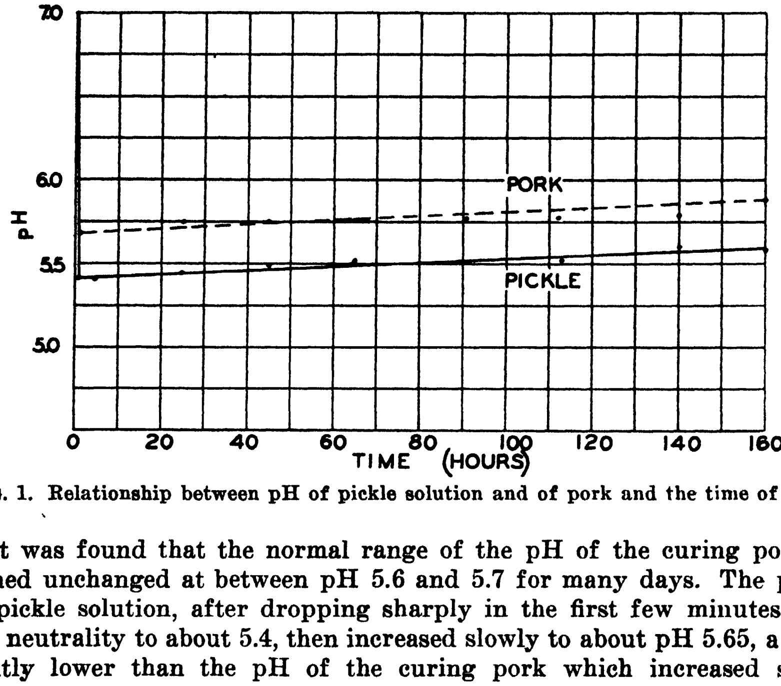

FIG. 1. Relationship between pH of pickle solution and of pork and the time of cure.

It was found that the normal range of the pH of the curing pork remained unchanged at between pH 5.6 and 5.7 for many days. The pH of the pickle solution, after dropping sharply in the first few minutes from near neutrality to about 5.4, then increased slowly to about pH 5.65, a value slightly lower than the pH of the curing pork which increased slowly throughout the period of observation.

It was possible to change the pH of the curing pork and the pickle and still approximate these normal pH relationships between the pork and pickle by curing in buffered solutions. A series of acetate, phosphate, and ammonium buffer solutions, covering a pH range from pH 2 to 10.5, were prepared from the solutions given (Table 1) and added to the pickle

TABLE 1

Concentration of Buffer Compounds

Compound	Normality
CH_3COOH	4 N
CH_3COONa	2 N
K_2HPO_4	2 N
KH_2PO_4	2 N
NH_4OH	4 N
$(NH_4)_2SO_4$	4 N

solution. The resulting changes in ionic strength of the pickle solution from its normal value of 4.14, varied from 3.61 to 4.59. However, subsequent spectrophotometric results on pork cured at pH 5.60 with a buffered pickle solution at an ionic strength of 3.52 and the unbuffered pickle of ionic strength 4.14, indicated that this difference in ionic strength had no effect upon the relationship between the pH and color. Furthermore, this experiment indicated that the phosphate buffer salts added to the pickle

TABLE 2

Effect on pH of Cured Pork of Addition of Two Milliliters of Buffer Solution to Pickle[1]

Buffer	Molar ratio	Original pH of pickle	After 1-hour cure		After 24-hour cure		After 9-day cure		Original pH of pork
			Pickle pH	Pork pH	Pickle pH	Pork pH	Pickle pH	Pork pH	
HAc / NaAc	32-1	2.90	3.60	4.85	3.95	4.60	4.50	4.60	5.40
HAc / NaAc	1-1	4.50	4.60	5.10	4.70	5.15	4.90	4.90	5.40
HAc / NaAc	1-32	6.00	5.50	5.45	5.50	5.60	5.50	5.65	5.40
Control	....	6.95	5.30	5.50	5.35	5.50	5.50	5.55	5.40
KH_2PO_4 / K_2HPO_4	32-1	4.10	4.60	4.60	5.00	5.60	5.10	5.45	5.75
KH_2PO_4 / K_2HPO_4	1 1	5.60	5.80	5.90	5.90	5.90	5.80	5.90	5.75
KH_2PO_4 / K_2HPO_4	1-32	8.15	7.00	5.95	6.85	6.05	6.70	6.70	5.75
Control	....	6.95	......	......	5.60	5.80	......	......	5.75
NH_4OH / $(NH_4)_2SO_4$	32-1	9.45	8.85	6.45	8.05	6.05	7.00	7.05	6.20
NH_4OH / $(NH_4)_2SO_4$	1-1	8.50	7.95	6.35	7.20	6.50	6.55	6.50	6.20
NH_4OH / $(NH_4)_2SO_4$	1-32	7.30	5.90	6.05	5.80	5.95	5.80	5.90	6.20
Control	....	6.95	5.50	5.75	5.57	5.80	5.75	5.80	6.20

[1] Size of samples—20 grams, one-inch cubes; pickle solution used—25 ml. pickle solution plus two ml. buffer solution; pH of unbuffered pickle solution, 6.95; temperature, 5°C.(41°F.); control of microorganisms—30 minutes under General Electric Germicidal Lamp; salinometer reading of pickle—82.5° at 25°C.(77°F.).

had no effect on the pH color relationship. Similar experiments proved that neither the acetate buffer solutions, the ammonia buffer solution, nor various acid bases and salts including HCl, HNO_3, tartaric, lactic, succinic, propionic, phosphoric, and acetic acids, sodium bicarbonate, sodium bisulphate, and sodium hydroxide in various concentrations had any specific effect on color independent of pH.

By this method of buffering the pork, it was possible to adjust the pH of the curing pork to an almost constant value in the pH range of 4.4 to 9

within one hour, even when using only two ml. of buffer solution and 25 ml. of pickle solution (Tables 2 and 3). The pH of the pickle also adjusted itself to near constancy in one hour as shown (Table 2). For practical purposes a mixture of eight milliliters of buffer solution and 25 milliliters of pickle solution gave the best results.

Owing to the rapid change in the pH of the pork to near constancy, it might be assumed that the nitric oxide myohemoglobin was formed in the pork at this near constant pH and that this was the pH at which color fixation was taking place.

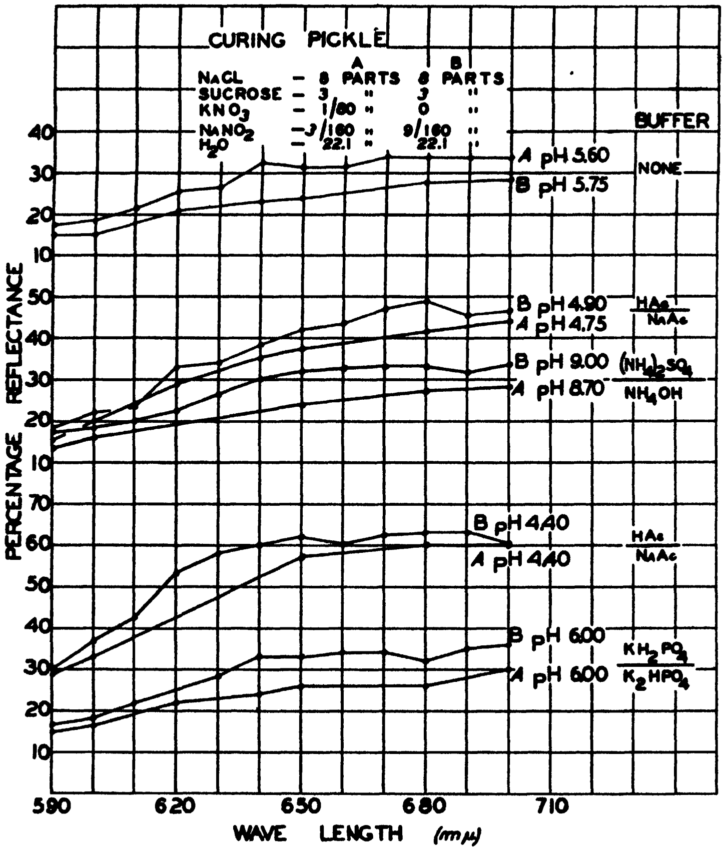

FIG. 2. Comparison of reflectance curves of pork samples cured with pickles containing different amounts of nitrite.

Samples of buffered pork cured in the described manner were cut in half and differences in color were determined as the differences in percentage reflection on the cut surface. In every case pH was also determined, and in some cases the moisture, nitrite, and chloride contents of the pork were determined in order to obtain an idea of the effect of pH on the diffusion of pickle ingredients, and in order to check the possibility of any effect on color by pH being indirectly caused by a direct effect on the curing salts. The results (Fig. 2) indicate a relationship between pH and color at

a pH of below 5, resulting in insufficient color fixation between pH 4.4 and 4.9. Results (Table 3) indicate a sharp drop in the nitrite content of the cured pork below pH 5, and an apparent linear relationship between the moisture content and the pH. The pH and the chloride content of the cured pork did not appear to be related.

In order to study the effect of the nitrite content of the pickle solution on the reflectance of the pork cured at the various pH's, duplicate sets of buffered pork cubes were cured, using the described pickle solution and a

TABLE 3

Effect of pH on Nitrite, Chloride, and Moisture Content of Cured Pork[1]

Buffer	Molar ratio	Moisture content of cured pork	Sodium nitrite content of cured pork	Chloride content of cured pork (dry basis)	pH after 24-hour cure		pH of original pickle
					Pickle	Pork	
		pct.	p.p.m.	pct.			
HAc/NaAc	32-1	59.4	1	22.7	3.70	4.40	2.75
HAc/NaAc	1 1	63.0	136	22.3	4.65	4.90	4.20
HAc/NaAc	1-32	67.3	273	23.5	5.75	5.75	5.50
KH_2PO_4/K_2HPO_4	32-1	64.0	375	19.8	4.85	5.50	4.00
KH_2PO_4/K_2HPO_4	1 1	67.3	333	23.7	6.15	6.00	5.95
KH_2PO_4/K_2HPO_4	1-32	66.6	375	19.0	8.15	6.45	8.90
$(NH_4)_2SO_4$/NH_4OH	32 1	67.3	375	23.1	6.00	5.70	7.60
$(NH_4)_2SO_4$/NH_4OH	1-1	69.0	200	23.6	8.50	6.50	8.80
$(NH_4)_2SO_4$/NH_4OH	1 32	69.8	188	23.2	9.25	9.00	9.95
No buffer	....	65.0	200	23.9	5.50	5.75	6.95

[1] Size of samples—20 grams, one-inch cubes; pickle solution used—25 ml. pickle solution plus eight ml. buffer solution; pH of pickle, 6 95; pH of fresh pork, 5.65; temperature, 5°C.(41°F.); control of microorganisms—30 minutes under General Electric Germicidal Lamp; salinometer reading of pickle—8.25° at 25°C.(77°F).

pickle containing no nitrate but three times the normal amount of nitrite. The results (Fig. 2) indicate no significant color difference in the meat cured in the two pickle solutions.

Taking a wave length 700 mμ as typical, the relation between the pH and the reflectance is shown (Fig. 3).

In order to determine in a limited way the applicability of these results to large cuts of cured meat, two four-pound Boston butts were cured, one with unbuffered pickle solution and the other with phosphate buffered pickle solution. It was impractical to control microorganisms since frequent pH analyses of the pickle were made and because of the length of time

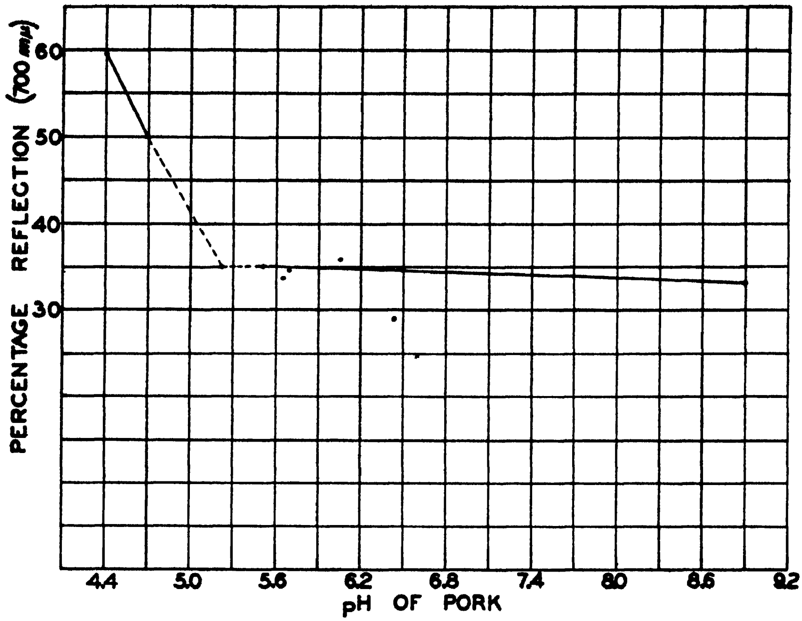

FIG. 3. Relationship between pH and reflection at 700 mμ.

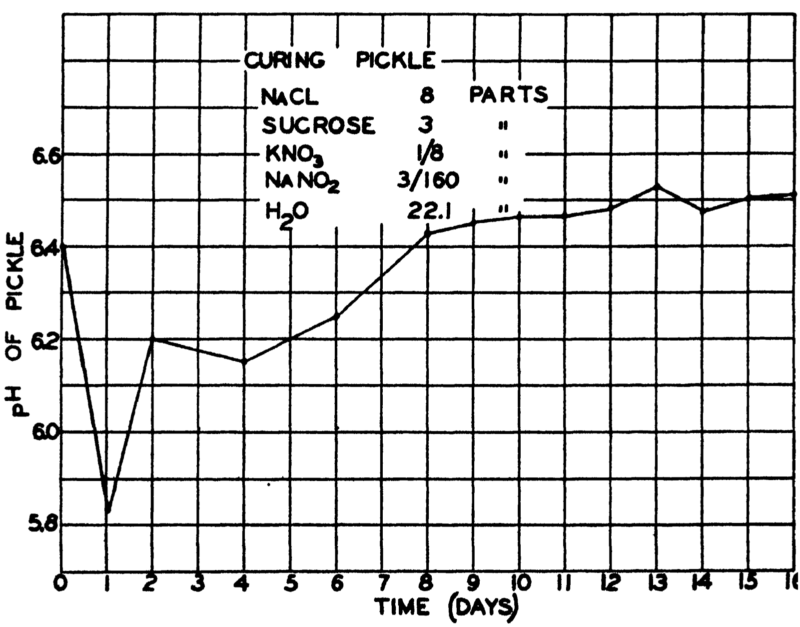

FIG. 4. Relationship between pH of pickle solution and time of curing a Boston butt.

required for the cure. The changes in pH of the pickle during curing are given (Fig. 4), and a comparison of the reflectance curves of small cured pork samples and the large cured samples (Fig. 5) indicates the two to be similar, at least over the limited pH range studied.

DISCUSSION

Owing to the high protein content of meat, it is very difficult to control its pH during cure. It is due to the buffering action of curing meat that the pH of unbuffered pickle solution falls to near that of the pork. It is probable that the pH of the pickle falls to slightly below that of the pork because of the effect of the high ionic concentration of the pickle solution on the pH of the protein buffers in the meat.

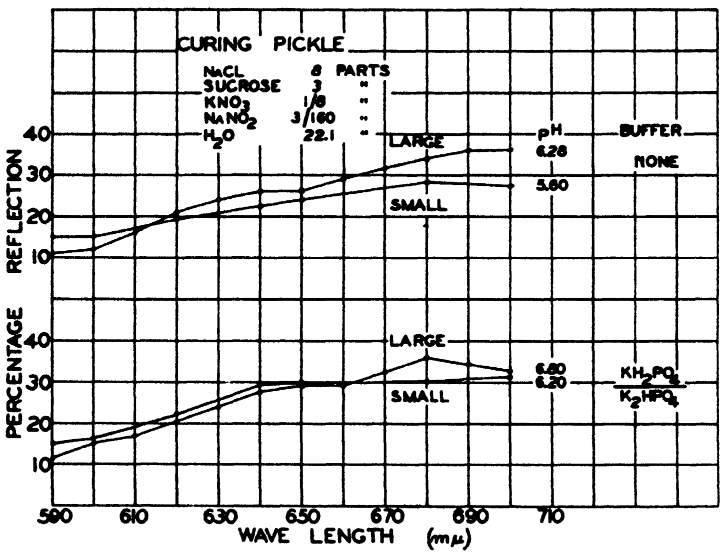

Fig. 5. Comparison of reflectance curves of large and small cured pork samples.

From the reflectance curves it is evident that above a pH of 5 there is no relationship between the pH and the color of cured meat. Between a pH of 4.4 and 4.9, color fixation is inadequate and at pH below 4 the brownish-grey color and lack of nitrite indicate no color fixation.

In the light of the chemistry of myohemoglobin and nitric oxide myohemoglobin and the results of the nitrite analysis of the cured pork, it appears that the pH plays an indirect role in the color development of cured pork.

It is well known that the reddish color of cured meat is due to the nitric oxide myohemoglobin formed by the reaction between myohemoglobin and nitrite. At a pH of 4.4 or below, the nitrite content of the meat is negligible and, therefore, there can be no color fixation.

This destruction of nitrite is due to the instability of nitrous acid which is formed in acid solution. However, at a pH of 4.9 the nitrite content of the cured meat, 136 parts per million, is many times in excess of the amount needed for proper color fixation, nevertheless color fixation is insufficient.

This indicates that the myohemoglobin of the meat is in some way decolorized or destroyed at this pH. This reaction may be analogous to the conversion of oxyhemoglobin to methemoglobin by dilute acids.

From these results it is apparent that it is impossible to obtain proper color fixation below a pH of 5. It was further noted that other qualities of meat cured at a pH less than 5 were undesirable; the meat became less tender with decreasing pH, probably owing to denaturation of the meat proteins, the moisture loss or shrink increased, and the flavor became undesirable and sour.

At pH's between 5 and 6, color fixation was normal and the meat was tender and palatable. At pH's alkaline to pH 7 the color fixation was normal, but the meat became tender to point of undesirable softness in some cases. In the case of pork cured with pickle which had been made alkaline with NaOH to a pH above 8, the meat became almost gelatinous in consistency after two or three days of curing.

The similarity between the reflectance curves of the small cured samples and the Boston butts indicates that the conclusions reached regarding the small pieces are applicable to larger pieces cured for many days, at least between the pH's of 6.3 and 6.8.

In conclusion, it may be stated that small pork samples cured in the presence of sufficient nitrite at pH's above approximately 5.2 will develop a normal desirable color. A curing range between pH 5.2 and 6 would appear to give optimum results. Below pH 5 color fixation is insufficient and the meat is tough, and above pH 7.5 the meat seems to change in properties toward a gelatinous-like consistency. Curing in the range of neutrality is inadvisable owing to the low rate of reaction between nitrite and hemoglobin, Brooks (1934), and because this pH range is favorable for the growth of many proteolytic bacteria. It does not seem practicable, therefore, to enhance the color of normal cured pork by alteration of the curing pH.

The results of this investigation on small cured pork samples are in agreement with the results of Urbain and Jensen (1940) on buffered solutions of nitric oxide hemoglobin. In view of the results obtained in the present study it would appear that the results of Winkler, Hopkins, and Thistle (1940) on Wiltshire bacon might also be interpreted to confirm the findings of Urbain and Jensen (1940).

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NUTRITIVE VALUE OF MILK PROTEIN[1]

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The nutritive value of a protein depends on two factors, its digestibility and its biological value. The digestibility is defined as the percentage of ingested protein which is absorbed from the intestinal tract, while the biological value is the percentage of the absorbed protein which is retained by the animal being nourished. Conditions for making these measurements have been stated by Martin and Robinson (1922) and Thomas (1930). Techniques for rats have been discussed by Mitchell (1924); Mitchell, Burroughs, and Beadles (1936); Johnson, Hogan, and Ashworth (1936); and French, Routh, and Mattill (1941).

Fairbanks and Mitchell (1935) have studied the quality of the protein of dried skimmed milk in relation to differences in the method of drying the milk. Henry, Houston, Kon, and Osborne (1939) have studied effects of evaporation and sterilization by the British process. The present study deals with five factors which might be expected to influence the protein quality of American evaporated milk. They are (1) the process of evaporating, irradiating, and canning; (2) the storage of canned milk; (3) the storage of raw milk as influenced by time, temperature, and preservatives; (4) the quality and quantity of protein fed the cow producing the milk; and (5) the stage of lactation of the cow producing the milk.

EXPERIMENTAL PROCEDURE

The original method of Mitchell (1924) for measuring digestibility and biological values was used with slight modifications. Relative effects of the previous history of the rats were minimized by feeding concurrently the milk being compared and by exchanging rats used for successive tests of the different kinds of milk. The 10 per cent sucrose in Mitchell's diet was replaced by 10 per cent lactose because lactose would be a significant part of all the supplements used. Collection periods of four days were used to avoid the use of the additional protein egg white, and because the rats were in much more normal condition after one week on the basal diet than on the 10 days needed for three preliminary days and a collection period of seven days. Water-soluble vitamins were supplied as Parke-Davis vitamin B complex and also as an extract with 90 per cent ethyl alcohol of Anheuser-Busch dried brewers yeast Strain C-50.

Evaporating, Irradiating, and Canning: The first step in the study of Factor 1 was to obtain from a unit batch of milk samples that were (1) packed raw in solid carbon dioxide, (2) evaporated and canned, and (3) evaporated, irradiated, and canned. The raw milk was mixed carefully in the tank and the raw-milk samples withdrawn and immediately iced. The remainder was put through the plant as a unit batch. The batch was

[1] Contribution No. 266, Department of Chemistry.

forewarmed, evaporated, and run into a drop tank. A sample was withdrawn from this tank, standardized by the addition of water only, homogenized, canned, and sterilized. The remainder of the batch in the drop tank was irradiated, standardized, homogenized, canned, and sterilized in a manner similar to the unirradiated sample. Similar parts were obtained from a second batch of raw milk two weeks later.

A group of 18 rats four weeks old were fed the basal nitrogen-free diet for a preliminary period of three days and for the first collection period of four days. The rats were then divided into three groups to equalize sexes, body weight, and appetite. Each group was then fed one of the three parts of the first sample of milk for a preliminary period of three days and for the second collection period of four days. The basal ration was decreased by the weight of milk solids fed and enough milk was fed to supply 8.5 per cent protein in the total ration.

After a second determination of nitrogen excretion on the basal diet, each group of rats was fed a second part from the second sample of milk, and the nitrogen excretion was measured. Each group of rats was then fed the third part from the second sample of milk and the nitrogen excretion measured. Nitrogen excretion on the basal diet was then measured at the end of this experiment on this factor. Two similar comparisons of raw and evaporated milk were made later on samples from two other plants.

Other comparisons of evaporated milk irradiated to 540 and 800 units of vitamin D per quart were compared with contemporary samples from the same plants, irradiated to contain smaller concentrations of vitamin D. The numbers of milk plants, rats, and milk samples used are shown (Table 3).

A group of 12 commercial brands of evaporated milk were also compared with average values for the experimental samples of evaporated milk and with average values for the samples of fresh milk tested under studies of all five factors.

Storage of Canned Milk: A series of tests similar to the first series studying Factor 1 began the study of Factor 2. Three samples of evaporated milk from a single plant were produced at different times so that the ages when tested were fresh, three months, and nine months. A new sample of freshly canned milk was provided after the second period on the basal diet. New cans of milk of each age were opened at the beginning of each feeding period. Later these tests were repeated on another set of samples from the same plant and on a set of samples from another plant where a sample 14 months old was used in place of the sample nine months old. Data obtained in studying Factor 1 also permit one comparison of freshly canned irradiated milk with a sample produced nine months earlier at the same plant.

Storage of Raw Milk as Influenced by Time, Temperature, and Preservatives: Since the raw milk used in studying Factor 1 was frozen during transportation to the laboratory, it was desirable that any effects of such storage on protein quality should be known. The first studies of Factor 3 were, therefore, comparisons between fresh raw milk and raw milk that had been frozen at —15°C.(5°F.) for two or three weeks. Comparisons of fresh raw milk with the same and similar milk after storing

one or two weeks at 5°C.(41°F.) were also made. In addition, effects of preserving the milk during test with formalin or formalin plus hydrogen peroxide were studied. For these tests one milliliter of 40 per cent formalin was added to each quart of an aliquot of the sample of fresh raw milk. After standing about one hour, three milliliters of superoxol per two quarts were added to half of the aliquot treated with formalin.

TABLE 1

Response of Rats to Milk, Either Raw (R), Irradiated Evaporated (I), or Unirradiated Evaporated (U) as Source of Protein

Rat No.	True digestibility			Biological value		
Period	2	4	5	2	4	5
1	86.5	94.2	93.8	100.0	100.0	90.7
2	93.4	93.0	99.5	95.8	94.0	74.6
3	87.2	96.0	92.0	82.9	91.3	75.6
4	98.7	100.0	94.2	86.2	96.2	83.6
5	89.3	97.6	95.3	86.8	92.5	75.0
6	93.7	99.2	86.8	90.8	100.0	71.0
Average	91.5	98.3	93.6	90.4	95.7	78.4
Milk	R	U	I	R	U	I
7	80.8	93.0	80.4	92.8	95.7	80.4
8	85.7	98.0	100.0	88.0	97.0	100.0
9	87.9	94.4	91.6	88.6	94.6	76.0
10	83.9	100.0	100.0	71.4	94.7	77.0
11	91.4	100.0	93.5	84.3	94.7	71.2
12	88.4	100.0	90.8	81.8	89.9	72.2
Average	86.4	97.6	92.7	84.5	94.4	81.4
Milk	I	R	U	I	R	U
13	80.8	98.2	90.0	92.9	98.5	79.6
14	87.3	100.0	91.7	84.6	96.0	74.8
15	90.0	91.4	92.0	98.3	99.7	89.9
16	87.7	96.1	95.0	80.4	89.8	70.7
17	81.2	98.4	94.0	70.6	93.7	66.6
18	99.6	100.0	90.7	85.7	93.8	80.9
Average	87.8	97.4	92.2	85.4	95.3	75.9
Milk	U	I	R	U	I	R

Quality and Quantity of Protein Fed the Cow Producing the Milk: For study of this factor one typical cow was selected from each of three experimental groups in the Kansas State College dairy herd. Each cow selected was in the second half of her second lactation on the diet studied. The diet of the first cow included only Atlas Sorgo and common salt. Quantities of grain fodder and silage were adjusted to make the best possible ration from this plant. The diet of the second cow included, in addition, bone meal and cottonseed meal. The diet of the third cow included alfalfa, wheat bran, and sorgo silage. The first two cows gave evidence of seriously inadequate nutrition. The protein concentration of milk from cows on the first diet was down 11 per cent the first year and

17 per cent the second year. The group of cows on the second diet produced milk with a normal protein concentration during the first lactation and eight per cent less than the expected concentration during the second lactation.

Stage of Lactation of the Cow: To study effects of the stage of lactation of the cow on the nutritive quality of the protein in her milk, nine samples of milk from five different cows were tested. Five of these samples were from cows within the second to sixth month of lactation, three samples were from cows in the second day of lactation, and one from a cow that had been lactating one week.

DISCUSSION OF RESULTS

The results of the first tests for effects of evaporation and irradiation (Factor 1) on digestibility and biological value are shown in considerable detail (Table 1). This table is typical of the extent and organization of tests for effects of each factor. The average value of each measure of quality for each kind of milk at each test period is also the basis for calculating the general average value for this measure of quality and its percentage difference from the corresponding value for the product used as a basis of comparison.

To illustrate, near the upper left corner of Table 1 under digestibility for Period 2 is the average 91.5 for raw milk. This value is the first entry in Table 2 and is averaged with averages from two other groups of rats

TABLE 2

Average Results of First Three Tests on Protein Quality in Raw Milk (R), Irradiated Evaporated Milk (I), and Unirradiated Evaporated Milk (U)

Kind of milk	Digestibility			Biological value		
	R	I	U	R	I	U
Group 1	91.5(2)[1]	93.6(5)	98.3(4)	90.4(2)	78.4(5)	95.7(4)
Group 2	97.6(4)	86.4(2)	92.7(5)	94.4(4)	84.5(2)	81.4(5)
Group 3	92.2(5)	97.4(4)	97.8(2)	75.9(5)	95.3(4)	85.4(2)
Average	93.8	92.5	92.9	86.9	86.1	87.5
Difference from R (pct.)	......	—1.4	—1.0	......	—0.9	0.7

[1] Numbers in parentheses refer to test period of rats.

for the general average 93.8. This general average is subtracted from similar general averages for irradiated and unirradiated evaporated milk. This difference is multiplied by 100 and divided by the general average for raw milk to obtain the percentage difference of the other kinds of milk.

These percentage differences together with corresponding differences for other tests and other factors are shown (Table 3). None of the differences shown in the last two columns of Table 3 are important.

The reason for expressing the values in Table 3 as per cent of a standard is to permit the averaging of tests made at different times and with different rats. Average direct values for the digestibility and biological value of fresh raw milk, milk evaporated under experimental conditions, and milk evaporated under commercial conditions are shown (Table 4).

TABLE 3

Influence on Quality of Milk Protein of Evaporation, Irradiation Storage in the Can, Storage of Raw Milk, Quality and Quantity of Protein Fed the Cow, and Stage of Lactation of the Cow

Milk being tested	Standard for comparison	Number of			Per cent difference from standard	
		Milk plants	Rats fed each milk	Milk samples	Digestibility	Biological value
Factor 1—Evaporation and Irradiation						
Unirradiated evaporated	Fresh raw	3	64	6	—1.5	—1.0
Irradiated 270 D/qt.	Fresh raw	1	18	2	—1.4	—0.9
Irradiated 540 D/qt.	270 D	1	18	1	—0.8	—0.5
Irradiated 800 D/qt.	270 D	1	24	1	—0.4	—0.2
Irradiated 800 D/qt.	540 D	1	25	1	—3.1	—1.6
Factor 2—Storage of Evaporated Milk in Can						
3 months U	Freshly canned U	2	44	6	—2.2	—1.3
9 months U	Freshly canned U	1	30	4	—0.5	—1.2
14 months U	Freshly canned U	1	18	1	—4.2	—0.5
9 months I	Freshly canned I	1	18	1	—4.0	—1.3
Factor 3—Storage of Raw Milk						
2 weeks at —15°C.	Fresh raw	1	34	3	—0.2	1.1
3 weeks at —15°C.	Fresh raw	1	13	2	—2.4	0.5
1 week at 5°C.	Fresh raw	1	24	4	1.0	2.0
2 weeks at 5°C.	Fresh raw	1	24	4	0.5	—0.8
With formula 1 c.c./qt.	Fresh raw	1	18	3	—0.1	0.4
With formula 1 c.c./qt. + superoxol 1.5 c.c./qt.	Fresh raw	1	18	3	1.8	—0.7
Factor 4—Quality and Quantity of Protein Fed Cow						
Sorgo and cottonseed	Alfalfa, bran, and sorgo	1	30	5	—0.1	—0.2
Sorgo only	Alfalfa, bran, and sorgo	1	30	5	1.2	0.7
Factor 5—Stage of Lactation of Cow						
Colostrum	Normal	1	58	5	0.3	—0.6

TABLE 4

Averages of Digestibility and Biological Value for Fresh Raw Milk, Milk Evaporated Experimentally by Producers, and Evaporated Milk From Retail Commercial Stocks

Type of sample	Number of			Digestibility	Biological value
	Milk plants	Rats for 3 periods each	Milk samples		
Fresh raw	4	70	13	93.4	90.5
Experimental evaporated	7	120	18	90.9	87.8
Commercial evaporated	12	72	12	91.8	89.4

SUMMARY

Tests on rats have shown that milk with high-quality protein could be either (a) evaporated, canned, and sterilized or (b) evaporated, irradiated to either 270, 540, or 800 units of vitamin D per quart, canned, and sterilized without loss of protein quality as measured by digestibility or biological value.

It was also found that cows fed greatly different qualities and quantities of protein produced milk with protein of uniformly high digestibility and biological value.

Concurrent tests on evaporated milk produced at a given plant two weeks, three months, or 14 months before completion of the test showed no serious loss of digestibility or biological value of milk protein.

The digestibility and biological value of milk protein did not change if the milk was stored with formalin or formalin plus superoxol at 5°C. for two weeks or at about —15°C. for three weeks.

The digestibility and biological value of milk protein did not differ significantly whether the milk was produced on the second day of lactation or at later periods up to five months.

Average values of digestibility and biological value were 93.4 and 90.5 for fresh raw milk, 90.9 and 87.8 for experimentally produced evaporated milk, and 91.8 and 89.4 for commercial evaporated milk.

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FACTORS INFLUENCING THE FLAVORING OF PICKLES WITH WHOLE SPICES AND ESSENTIAL OILS[1]

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Spices are the chief source of flavors used in the pickle industry. With few exceptions the principal and characteristic flavor of a spice is contained in that component which is volatile with steam. This volatile component is termed the essential oil of the spice. When the spice is extracted in vinegar or combinations of vinegar and sugar, some of the essential oil is dissolved. The flavor is in this way made available to the pickles. The oils are practically insoluble in water and only slightly soluble in acetic acid solutions. Therefore, only a small portion of the spice oil is available when the spices are extracted in this way.

Essential oils from spices are often used, as such, to flavor pickles. The water-insoluble oil may be incorporated into the flavoring solution by any one of the following three generally accepted methods: (a) a solution of oil in a solvent such as ethyl alcohol is added directly to the spicing liquor; (b) the oil is "cut" with sugar or salt; (c) an oil-water emulsion is prepared and added to the liquor. When organic solvents such as alcohols or sugar are used, the emulsion which forms upon the addition of the solution to vinegar is unstable; that is, the oil globules coalesce rapidly, rise to the surface of the spicing liquor, and are partially lost by subsequent volatilization. Specially prepared emulsions are used to maintain the oil in suspension until the flavor is either dissolved by the pickle liquor or absorbed by the pickle.

This work deals with three aspects of pickle spicing, viz., the extracting efficiency of vinegar brines for whole spices, the location of oils in spiced pickles, and a study of the methods commonly used to incorporate oils in pickling solutions. Additional work is planned in order to make a more detailed study of certain phases of the problem in an attempt to clarify and amplify some of the questions still unanswered in this work.

EXTRACTION EFFICIENCY OF VINEGAR SOLUTIONS

The solutions used for extracting the spices were 130-grain vinegar (13 per cent acetic acid) and sucrose solution in 130-grain vinegar. The spices used were whole cloves, whole dill seed, cassia quills, and nutmegs. Cloves and dill seed were used in the whole form in the extraction and subsequent distillation. The cassia quills and whole nutmegs were broken into coarse pieces by means of a food grinder immediately before the extraction.. When finely ground spice was used, there was a tendency for it to stick to the bottom of the flasks and become charred. Whole or

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broken spice did not do this. Dill seed was extracted with 130-grain vinegar only.

The apparatus used to determine the volatile oil in the spices was the Clevenger apparatus as recommended by the Association of Official Agricultural Chemists (1934, 1939) for spices. Preliminary distillations were run on unextracted spices in which the effects of time, temperature of bath, quantity of water used, size of flask, and condition of the spice were studied. The effect of the length of time of distillation was different for different spices as shown (Table 1).

TABLE 1

Volatile Oil Obtained From Spices After Distilling for Various Lengths of Time

Spice	Per cent oil		
	5 hours	8 hours	11 hours
Allspice	2.7	3.0	3.0
Cassia	1.0	1.1	1.1
Cinnamon	1.0	1.1	1.1
Cloves	14.2	17.0	18.4

The Clevenger method was used throughout the experiment to determine the volatile oil, first in unextracted spices and then in extracted spices. The per cent volatile oil in various unextracted spices as determined in this experiment is shown (Table 2) along with those reported by Clevenger *et al.* (1933, 1934, 1935, 1941) and Gildemeister and Hoffman (1916).

TABLE 2

Volatile Oil Obtained From Spices by Different Investigations

Spice	Present study	Reported by	
		Clevenger	Gildemeister
	pct.	*pct.*	*pct.*
Allspice	2.7- 3.0	2.7- 4.3	3.0- 4.5
Cloves	17.0-18.4	18.0-18.2	16.0-19.0
Cassia	1.15	....	1.2
Dill seed	2.9	....	2.0- 4.0
Nutmeg	9.4	6.8- 8.7	7.0-15.0
Cinnamon	1.1	3.2- 3.3	0.5- 1.0

EXTRACTION EFFICIENCY OF VINEGAR

A study was also made of the extraction efficiency of vinegar under certain conditions. The influence of time and temperature and the addition of sugar to vinegar upon the amount of volatile oil extracted from spices was determined. Extractions were made on whole cloves with 130-grain vinegar and 130-grain vinegar plus 25 per cent sugar solutions at 93.3 and 65.6°C. (200 and 150°F.). The extraction at 200°F. was made by heating the flask containing the spice and solution to 200° and allowing it to cool immediately. The extraction mixtures at 150°F. were held at that temperature for four hours in a constant temperature water bath. Samples were then allowed to stand for one, seven, and 21 days before distillation.

Broken cassia and nutmegs were extracted in similar solutions at 150°F., only, for four hours. The nutmegs stood in the extracting solutions for one, 14, and 60 days before distillation. The dill seed was extracted in 130-grain vinegar and 65-grain vinegar at 200°F. for three hours (Tables 3 and 4).

At the proper time the extracting solutions were poured off; the spices were washed with two portions of cold water and then were distilled. The oil which was obtained in this distillation had not been extracted from the

TABLE 3

Essential Oil Extracted From Spices by Vinegar Solutions

Spice	Number of days extracted	Temperature treatment	Volatile oil: Original spice	Left in spice after extraction	Removed by vinegar at each extraction	Removed by vinegar and sugar at each extraction
		°F.	pct.	pct.	pct.	pct.
Cloves	1	200 [1]	17.0	14.4	2.6	.....
Cloves	7	200	......	13.6	3.4	.....
Cloves	21	200	......	15.6	1.4	.....
Cloves	1	150 [2]	17.0	12.0	5.0	.....
Cloves	7	150	......	16.0	1.0	.....
Cloves	21	150	......	15.2	1.8	.....
Cloves	1	200	17.0	12.8	.....	4.2
Cloves	7	200	......	14.4	.....	2.6
Cloves	21	200	......	16.8	.....	0.2
Cloves	1	150	17.0	15.6	.....	1.4
Cloves	7	150	......	14.4	.....	2.6
Cloves	21	150	......	16.0	.....	1.0
Cassia	1	150	1.2	0.3	0.9	.....
Cassia	7	150	......	0.4	0.8	.....
Cassia	21	150	......	0.3	0.9	.....
Cassia	1	150	1.2	0.5	.....	0.7
Cassia	7	150	......	0.4	.....	0.8
Cassia	21	150	......	0.3	.....	0.9
Nutmeg	1	150	9.4	6.8	2.6	.....
Nutmeg	14	150	......	6.8	2.6	.....
Nutmeg	60	150	......	7.6	1.8	.....
Nutmeg	14	150	9.4	6.4	.....	3.0
Nutmeg	60	150	......	7.6	.....	1.8
Dill seed	1	200	2.9	2.8	0.1	.....
Dill seed	1	200	......	2.9	0	.....

[1] Temperature of mixture brought to 93.3°C.(200°F.) after which spices stood in extracting solution at room temperature for number of days indicated in Column 2. [2] Mixture heated at 65.6°C.(150°F.) for four hours after which spices stood in extracting solution for number of days indicated in Column 2.

spice by the vinegar solution. The amounts of oil in per cent extracted by the solutions under all conditions are shown (Table 3). The effect of time of standing upon the extraction efficiency was not consistent; under one set of conditions, for example, the per cent oil extracted from cloves increased from 15 to 20 per cent in seven days but actually decreased to eight per cent in 21 days. When the effect of temperature and the addition of sugar were studied, it was observed that, while temperature alone did not appreciably affect the amount of oil extracted, the addition of sugar

decreased the amount of oil extracted from cloves and cinnamon, but slightly increased the amount extracted from nutmeg (Table 4).

In order to determine the efficiency of vinegar as an extracting agent cloves were repeatedly extracted with 130-grain vinegar. This was done by placing 25 grams of whole cloves and 100 ml. of 130-grain vinegar in flasks and heating them in a steamer to 200°F., after which they were

TABLE 4

Influence of Temperature and Addition of Sucrose on Extraction Efficiency of Vinegar

Spice	Solution	Temperature	Total oil extracted
		°F.	pct.
Cloves	130-gr. vinegar	200	14.7
Cloves	130-gr. vinegar	150	15.0
Cloves	25% sucrose in 130-gr. vinegar	200	13.5
Cloves	25% sucrose in 130-gr. vinegar	150	10.0
Cinnamon	130-gr. vinegar	150	73.0
Cinnamon	130-gr. vinegar with sucrose	150	67.0
Nutmeg	130-gr. vinegar	150	25.0
Nutmeg	130-gr. vinegar with sucrose	150	26.0

allowed to stand for one to 21 days with frequent changes of vinegar as indicated (Table 5). At each change the old vinegar was poured off and replaced with 70 ml. of new vinegar since the dry spice had absorbed and retained 30 ml. from the previous addition. At the end of the respective periods, the amount of oil left in the cloves was determined by distillation (Table 5).

TABLE 5

Volatile Oil Extracted From Cloves by Repeated Extractions With 136-Grain Vinegar

Number of times extracted	Total number of days extracted	Volatile oil left in cloves	Oil removed by this extraction	Total extracted
		pct.	pct.	pct.
0	....	18.4	0	0
1	1	16.8	1.6	6.5
2	2	16.8	0	6.5
3	3	16.8	0	6.5
4	4	15.2	1.6	17.5
5	10	14.4	0.8	22.2
7	15	12.8	1.6	28.2
9	21	9.6	3.2	47.8

In addition to the work above, three spices—dill herb, allspice, and cassia—were picked out of a mixture of whole spices consisting of mustard and celery seed, bay leaves, chili peppers, cardamom, ginger root, allspice, and cassia (with the latter two predominating) which had been used previously to make genuine dill pickles at a pickle plant. These three spices were tested for their volatile oil content. The results were as follows:

Spice	Per cent volatile oil	
	After use in making dill pickles	Normally present in unused spice
Dill herb	0.8	.25-.4[1]
Allspice	1.6	3.0
Cassia	1.3	1.2

[1] Yield from commercial steam distillation.

However, the odor of the oils as well as the amount obtained from these spices indicated that all the distillate obtained from them was not the original oil of that particular spice but rather a mixture of oils. This was especially true of the dill herb which distinctly was not dill oil but rather a mixture of spice oils, indicative that the dill herb acted as an excellent absorbent for spice oils.

LOCATION AND MECHANISM OF ABSORPTION OF VOLATILE OIL BY PICKLES

Whether the essential oils are extracted from the spices or added, as such, to the flavoring solution, they are present as small globules in the pickling brine. A preliminary experiment was performed in order to determine the form and location of these oil globules in spiced pickles. To 50 gallons of pickles, which had been sweetened but not spiced, was added enough spice oil, in emulsion form, to make the final concentration one part oil to 5,000 parts of pickle and liquor. After several weeks, samples of the liquor were examined miscroscopically for the presence of oil globules. None were found. A 1:5,000 dilution of spice oil in a fresh vinegar-sugar solution of the same composition contained oil globules which were easily found. Assuming a drop of the liquor containing 1:5,000 parts of oil to have a volume of .05 ml., and the size of the oil globules to have a diameter of 50 microns, then each drop of liquor would contain 153 globules of oil. If the oil globules were 10 microns in diameter, there would be 20,000 per drop. Since globules of this size would be easily discernible under the microscope, it was assumed that they had been either absorbed by the pickles or had evaporated.

It is well known that volatile oils are readily absorbed by some ether-soluble substances. A determination of crude fat was made on the epidermis, parenchyma, and the central portion (placental tissue and the seeds) of processed, unspiced pickles. The epidermis was scraped from the pickle with a sharp knife, and the placental tissue and seeds were cut and scraped from the remaining parenchyma. These tissues were then dried in flowing hot air at 68.3°C.(155°F.) for 12 hours, cooled in a desiccator, weighed into a Soxhlet extraction thimble, and extracted with an anhydrous ethyl ether. Two sizes of pickles were used for the extraction, namely, 4500-6000's and 1000's.[3] It was found that in the smaller pickles the central portion contained the greatest amount of crude fat, while in the larger pickles the epidermis contained most of the fat; these data are shown (Table 6).

[3] Pickle size is expressed as the number of pickles contained in a 45-gallon barrel.

The location of ether-soluble constituents having been determined, stains were made on whole pickles, cut pickles, and lamellar sections with Sudan IV and viewed under microscopes having magnifications from 9x to 450x. The following three procedures were used for staining:

(a) A pickle was allowed to stand in a concentrated solution of Sudan IV in dill oil for one week. When lamellar sections of this pickle were viewed under the microscope, it was observed that the epidermal layer of palisade cells was stained a very deep red. The skin at the blossom-end of the pickle was not as deeply stained as that at the stem-end. No internal areas were stained.

(b) A cut pickle was placed into a Sudan IV-dill oil mixture for 24 hours. The oil penetrated fairly well into the exposed central portion and was concentrated in the epidermis but was not apparent in the interior of the parenchymatous cells.

TABLE 6

Ether Extract of Epidermis, Parenchyma, and Central Portion of Processed Pickles

Size of pickles	Section	Whole pickle	Ether extract	Total ether extract of pickle
		pct.	*pct.*	*pct.*
4500-6000	Epidermis	30	3.5	38.5
	Parenchyma	53	1.9	36.5
	Central portion	17	4.0	25.0
1000	Epidermis	11	4.4	31.3
	Parenchyma	59	1.2	45.5
	Central portion	30	1.2	23.0

(c) Dried lamellar sections were placed into a Sudan IV-dill oil solution for 24 hours, washed in 95 per cent alcohol for 30 seconds, blotted, and dried. Similar sections were stained in alcoholic Sudan IV. The skin and vascular ducts in the center portions of the control pickle were stained lightly. The pickle which had been in the dill oil was stained heavily in the vascular ducts, in the nuclei of the cells just beneath the epidermis, as well as in the epidermis itself.

The above facts led to an experiment to determine the volatile oil content of pickles and pickle sections which had been treated with relatively concentrated dill-oil emulsions. Dill oil was used because it is very insoluble in water. In this experiment salt-stock pickles were processed to remove all of the salt. To these pickles enough water and dill-oil emulsion were added to give the desired final concentration of oil. Two different sizes of pickles were used, 6000-7500's and 1200-1600's. Final concentrations of oil used were one part to 100 parts pickle and brine, 1:200, 1:1,000, 1:2,000. The ratio of pickles to brine was five to three. After the pickles had remained in these solutions for three days, they were removed, washed, ground, and distilled in the Clevenger apparatus for the determination of volatile-oil content. A similar distillation of unspiced pickles revealed that they contained less than .05 per cent volatile oil. The results (Table 7) indicate that the size of the pickle did not

influence the amount of oil absorbed, and that a greater per cent of oil was absorbed by the pickles from the higher dilutions. Under the conditions of this experiment the pickles absorbed from a minimum of 18 to a maximum of 63 per cent of the oil added to the spicing liquor.

In the determination of the location of the volatile oil in the 1000-size spiced pickles two different procedures were used. In one set the pickles were peeled before they were spiced, and in the other set the pickles were peeled after they were spiced. The concentration of spice oil used was 1:200. The ratio of pickles to brine was two to one. The results of this experiment (Table 8) show that most of the oil was absorbed by the epidermis of the pickle.

TABLE 7

Relation Between Concentration of Dill Oil Present and Amount Absorbed by Processed Pickles

Ratio of oil to pickles and liquor	Size of pickles	Total oil added	Oil absorbed	Oil absorbed —based on oil absorbed from 1:100 dilution	Amount absorbed of that present in brine
		ml.	*ml.*	*pct.*	*pct.*
1:100	6000-7500	8.0	1.10	100	14
1:200	6000-7500	4.0	1.00	90	25
1:1,000	6000-7500	0.8	0.50	45	63
1:100	1200-1600	8.0	1.45	100	18
1:200	1200-1600	4.0	1.20	83	30
1:1,000	1200-1600	0.8	0.50	34	63
1:2,000	1200-1600	0.4	.22-.25	17	56-63

This work was continued to determine how much of the oil in the pickle was actually in solution. Pickles were treated as before with emulsions of dill oil. The ratio of pickle to brine in this experiment was five to three. As much fluid as possible was removed from the pickles by a hand press, and the pulp from the pickles was distilled in the Clevenger apparatus to determine the amount of oil retained in the pulp. The results (Table 8) indicate that most of the oil was retained within the solid portion of the pickle. The data (Tables 6, 7, and 8) would indicate not only that the greater amount of dill oil was absorbed by the pickle from the liquid but that it was dissolved in those tissues of the pickle containing the greatest amounts of lipids. Since 37 per cent of the pickle was removed as juice,

TABLE 8

Location of Dill Oil Absorbed by 1000-Size Pickles

Section	Whole pickle	Total oil absorbed
	pct.	*pct.*
Epidermis	27	53-58
Parenchyma	17	27-29
Central portion	56	15-18
Pressed pulp	63	89

63 per cent of the pickle actually held 89 per cent of the oil absorbed by the whole pickle.

METHODS OF INCORPORATING SPICE OIL INTO PICKLES

Four methods of incorporating oil of dill in spicing liquors were studied, namely, the addition of (a) an alcohol solution of oil, (b) an emulsion, (c) an oil-sugar mixture, and (d) the plain oil itself. In each case 3.6 ml. of oil of dill was added to a gallon jug containing 2,200 ml. of processed pickles and 1,400 ml. of acidified water. The oil in alcohol solution used was one part oil in nine parts of alcohol. A gum-arabic emulsion was used containing 25 per cent oil and 10 per cent gum. Duponol PC, a surface tension depressant in which the active ingredient is sodium lauryl sulfonate, was added to one of the samples in which the plain oil was incorporated. The sugar-oil mixture was made by the addition of 3.6 ml. of oil to 400 grams of sugar, and the addition of this mixture to 550 ml. of 136-grain vinegar and water to make 1,400 ml. After the sugar was dissolved the liquor was added to the jug of pickles. Trichloracetic acid

TABLE 9

Oil of Dill Absorbed by Pickles From Acidified Solutions (One Part Oil to 1,000 Parts Pickles and Brine)

Method of incorporation	Absorbed
	pct.
Alcohol solution	61
Emulsion	55
Plain oil	62
Plain oil with Duponol PC	55
Sugar-vinegar	63

was used to acidify the samples not containing vinegar. *The jugs were inverted and shaken daily.* This gave a better dispersion of the oil and a more intimate contact with the pickles than would be obtained in plant practice unless the tanks were pumped or the barrels rolled daily. After two weeks the pickles were drained, ground with 1,000 ml. of water, and distilled in the Clevenger apparatus. The results (Table 9) indicated that under the conditions of this experiment no one method incorporated very much more oil than any other method. It was observed during the course of the experiment, however, that the oil came to the surface of the brine in all of the jugs except the one in which the emulsion was used.

An attempt was made to determine how much of the oil added in alcoholic solution to various types of pickling liquors would return to the surface of the liquor. A 10-per cent dill oil in alcohol solution was added to (a) water, (b) 136-grain vinegar, (c) 50 per cent sucrose in 136-grain vinegar, (d) 20 per cent sodium chloride in 136-grain vinegar, and (e) 50 per cent sucrose and 15 per cent sodium chloride in 136-grain vinegar. Ten ml. of the alcoholic solution were added to 90 ml. of the above solutions in a 110-ml. cassia flask. The mixtures were shaken and allowed to stand until oil was visible on the surface of the solution. The oil layer was brought up into the neck of the flask by the addition of the respective

solutions, where it was measured. The results (Table 10) show that the salt-sucrose-vinegar solution seemed to retain the oil better for a period of two days after which time there was a noticeable increase in the amount of oil separated.

TABLE 10

Stability of One Per cent Alcohol-Dill Oil Emulsions in Various Types of Pickling Brines

Type of brine	Oil emulsified	At end of
	pct.	
Water	10	5 minutes
136-grain vinegar	20	15 minutes
50% sucrose in 136-grain vinegar	30	15 minutes
20% NaCl in 136-grain vinegar	15	5 minutes
50% sucrose and 15% salt in 136-grain vinegar	45	30 minutes
50% sucrose and 15% salt in 136-grain vinegar	40	16 hours
50% sucrose and 15% salt in 136-grain vinegar	15	48 hours

SUMMARY

The greatest amount of oil was extracted by vinegar from the spices in 24 hours, after which time the oil was dissolved from the spices at a much slower rate under the conditions of the treatment used. The addition of sugar to vinegar decreased slightly the amount of oil extracted from cloves and cinnamon but had little effect on nutmeg. Vinegar is a poor solvent for clove oil, since nine extractions over a period of 21 days removed only 47.8 per cent of the oil from the cloves. This indicates a tremendous loss of oil by the use of whole cloves in pickle manufacture.

Processed pickles absorbed from 56 to 63 per cent dill oil from a spicing brine containing 1:1,000 and 1:2,000 dilutions of the oil. A greater amount of oil was absorbed from processed pickles from the higher than from the lower concentrations. However, a relatively greater per cent of oil was absorbed from the lower than from the higher concentrations. In the stronger concentrations (1:100 and 1:200) the amount of oil absorbed was not directly proportional to the amount of oil present, while at the weaker concentrations (1:1,000 and 1:2,000) it was directly proportional.

The per cent of ether extract varied inversely with the size of the pickle, the smaller pickles having a greater amount than the larger. There was variation of the amount of ether extract in the same type of tissue in different size pickles.

The greatest concentration of dill oil in 1000-size pickles was found in the epidermis. It was also found that in this size of pickle the epidermis contained the greatest amount of ether-soluble materials. It would, therefore, appear that there is a direct correlation between the amount of ether-soluble materials and the amount of essential oils absorbed by the pickle.

The different methods tried so far for incorporating a 1:1,000 dilution of dill oil into pickles within a period of two weeks showed little difference in the amount absorbed.

An emulsion resulting from the addition of a 10-per cent solution of dill oil in 95-per cent alcohol to various types of pickling solutions showed the greatest stability in a brine containing 50 per cent sucrose, 15 per cent salt, and 136-grain vinegar, and the least stability in a brine containing 20 per cent salt and 136-grain vinegar.

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LINE-SPREAD AS AN OBJECTIVE TEST FOR CONSISTENCY

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Various elaborate instruments are available for determining the consistency of food-products by measuring their viscosity and penetrability. In a study in this laboratory an unusually simple and inexpensive device was employed which measures consistency in terms of the ability of products to spread.

A flat glass plate is put on a surface checked for evenness with a spirit level; beneath is a diagram of concentric circles an eighth of an inch apart, the smallest with a diameter of two inches. This circle has no number but the second one is numbered "1" and the others numbered outward in order. Several concentric-ring diagrams placed on the same chart permit the rapid testing of samples. A hollow cylinder, the exact diameter of the innermost circle, is also required. In this laboratory it was three-eighths of an inch deep and was made by removing the handle from a biscuit cutter.

To measure the spread of the food, called line-spread, the cylinder is placed on the glass directly above the smallest circle over which it fits exactly; it is filled with the material to be tested, leveled off with a spatula, and carefully lifted. The product is then allowed to spread for exactly two minutes, after which time readings are taken at four widely separated points that mark the limits reached by the substance. The average of the four readings is recorded as the line-spread of the sample. It represents the number of eighth-inch units a given volume of material at room temperature has spread in a two-minute period.

EXPERIMENTAL PROCEDURE

Applesauces were made by a standardized procedure from different varieties of apples and were tested over a period of several years, Pfund (1939). A group of judges rated the sauces on several qualities, including consistency and juiciness. The correlation coefficients between the line-spreads of applesauces and the personal-opinion ratings of consistency and juiciness were determined.

Cream fillings were made with a fixed proportion of flour, sugar, milk, flavoring, and either whole egg, egg yolk, or egg yolk and lemon juice, Grawemeyer (1942). They were cooked ½, 1½, 2½, and 3½ minutes after the egg was added. Their consistencies were measured by a Precision penetrometer, a Stormer viscosimeter, and the line-spread device. Differences in consistency as noted when tested organoleptically were recorded. Mean differences between the values for fillings cooked for varied lengths of time were calculated from the data of each of the three objective tests.

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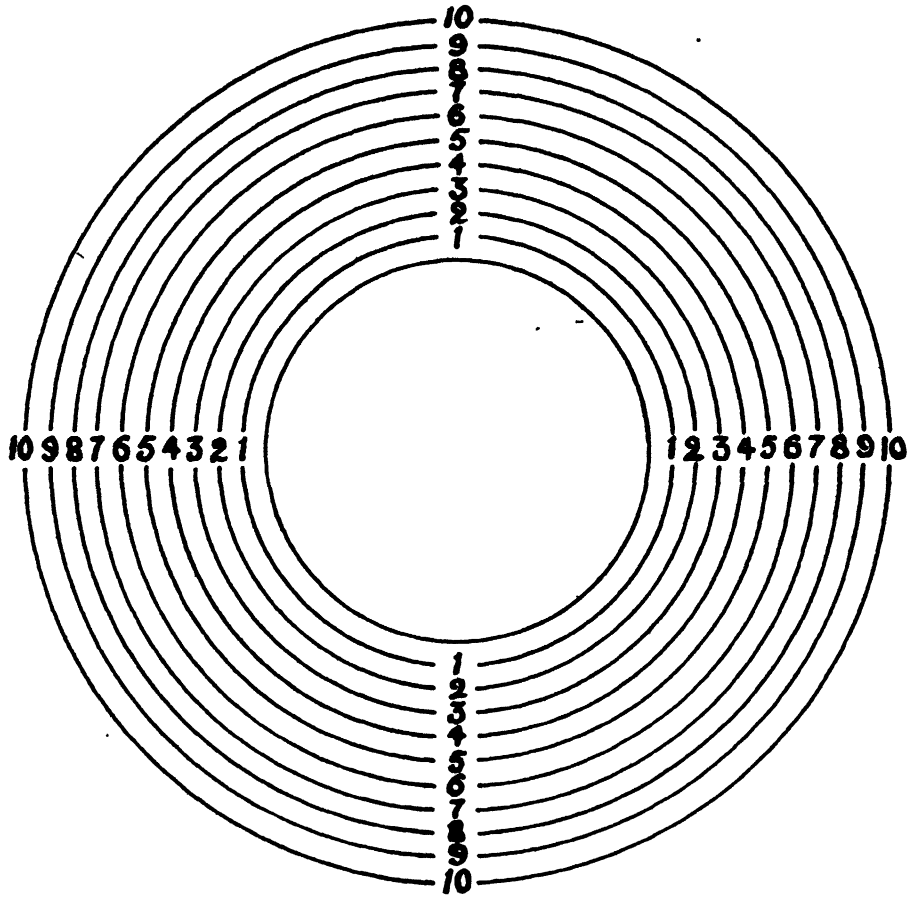

FIG. 1. Diagram of concentric circles used beneath a glass plate to measure consistency of foods in terms of line-spread.

Student's "t" was used to determine the mathematical significance of these differences.

DISCUSSION OF RESULTS

In the studies on applesauces it was found that those most frequently rated thin gave higher line-spreads than those rated thick (Table 1). Those

TABLE 1

Line-Spread and Personal-Opinion Ratings of Consistency of Applesauces[1]

Line-spread eighth-inch units	Number of opinions	Consistency ratings of applesauces (per cent of opinions)			
		Thick	Medium	Thin	Total
Less than 3.0	103	57	43	....	100
3.0 to 3.9	231	46	53	1	100
4.0 to 4.9	276	25	73	2	100
5.0 to 5.9	205	19	68	13	100
6.0 or more	165	15	49	36	100

[1] Twenty-eight varieties, 980 opinions.

most frequently rated very juicy gave higher line-spreads than did those rated dry (Table 2).

TABLE 2

Line-Spread and Personal-Opinion Ratings of Juiciness of Applesauces[1]

Line-spread eighth-inch units	Number of opinions	Juiciness ratings of applesauces (per cent of opinions)				
		Very juicy	Juicy	Slightly dry	Dry	Total
Less than 3.0	136	1	23	28	48	100
3.0 to 3.9	253	1	37	41	21	100
4.0 to 4.9	287	3	49	37	11	100
5.0 to 5.9	210	10	72	10	8	100
6.0 or more	165	19	69	3	9	100

[1] Twenty-eight varieties, 1,051 opinions.

Although the frequency table shows an obvious trend, the correlation coefficients are small, .44 between line-spread and consistency and .50 between line-spread and juiciness. The relatively small correlation coefficients may indicate that for foods that vary in juiciness as well as in thickness, the latter quality cannot be judged independently by organoleptic means.

With cream fillings, juiciness is not involved. The thickness of the fillings increased in proportion to the length of time of cooking after the egg was added. This was indicated by personal-opinion ratings and was confirmed by line-spread values (Table 3). The standard deviations on

TABLE 3

Line-Spread of Cream Fillings[1]

Cooking time after eggs were added	Whole-egg filling	Egg-yolk filling	Egg-yolk lemon filling
	One-eighth-inch units		
min.			
½	5.5±.54[2]	4.7±.40	4.1±.19
1½	3.1±.16	2.5±.16	3.3±.09
2½	3.0±.17	2.1±.11	3.0±.20
3½	2.5±.14	1.5±.04	2.6±.16

[1] All values are averages of eight to 12 samples tested at room temperature. [2] Standard deviation of the mean.

the whole were small, thus the test was satisfactory for the 150 fillings made during a period of 16 weeks.

A larger number of significant differences were found between the series of fillings when tested with the line-spread device than with the penetrometer or the viscosimeter (Table 4). In almost all of the cases in which the judges noted differences in consistency, significant differences in line-spread were measured.

TABLE 4

Mean Differences and "t" Values From Objective Tests on Cream Fillings[1]

Cooking time after adding eggs		Viscosity[2]		Penetrability		Line-spread	
	min.	MD	"t"	MD	"t"	MD	"t"
Whole-egg filling	½ and 1½	9.9	4.68[3]	22.4	7.23[3]	2.40	4.14[3]
	1½ and 2½	5.3	2.77[3]	1.9	.82	0.07	0.57
	2½ and 3½	2.8	0.74	4.7	1.46	0.50	4.30[3]
	1½ and 3½	5.4	3.49[3]	11.6	3.93[3]	0.84	3.80[3]
Egg-yolk filling	½ and 1½	1.6	0.96	19.0	6.71[3]	2.17	5.40[3]
	1½ and 2½	No difference		1.5	1.71	0.48	3.60[3]
	2½ and 3½	1.9	1.88	6.9	7.11[3]	0.56	4.67[3]
	1½ and 3½	No difference		9.6	5.39[3]	1.10	6.42[3]
Egg-yolk lemon filling	½ and 1½	2.1	1.51	10.1	7.65[3]	0.80	3.04[3]
	1½ and 2½	No difference		No difference		No difference	
	2½ and 3½	No difference		3.8	2.25	0.50	1.60
	1½ and 3½	2.1	1.09	4.3	2.00	0.70	4.39[3]

[1] All values are averages of eight to 12 samples. [2] MD=mean difference; student's "t" $= \frac{MD}{\sigma MD}$.
[3] Odds greater than 99 to 1

SUMMARY

The consistency of applesauces and cream fillings was measured in terms of the ability of the products to spread. The simple and inexpensive line-spread device proved most satisfactory for it measured differences in consistency that were not shown by other instruments. The line-spread values obtained with cream fillings were comparable to penetrability and viscosity values.

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QUANTITATIVE DETERMINATION OF VITAMIN B_1 IN NAVY BEANS

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The purpose of this study was the determination of the vitamin B_1 content of baked beans. The small white navy bean *(Phaseolus vulgaris)*, called the Michigan bean in the Chicago market, was used.

The beans were baked at the conventional simmering temperature for many hours and are here compared with the higher temperatures and shorter periods of cooking of the boiled and baked beans prepared by Kelly and Porter (1941). The results may be interpreted as bearing on the comparison of different temperatures and times of cooking upon the vitamin B_1 value of cooked foods.

Data on the vitamin B_1 content of baked navy beans are limited. Determinations reported on the dried, uncooked beans as well as two figures for boiled and one for baked beans, all calculated back to the dry basis, and one determination of commercially canned beans as fed are shown (Table 1).

Reports on the vitamin B_1 value of cooked beans as compared with the original dry values are conflicting. Lantz (1938) reported some loss of thiamin in the cooking of pinto beans in tap water with or without soda with long cooking and high temperatures; however, Aughey and Daniel (1940) report that navy beans retained all of their original thiamin value when cooked in tap water with or without soda. They ground the raw beans to a powder before feeding. Kelly and Porter (1940) have reported that beans baked and boiled in distilled water have more available vitamin B_1 than when in the raw state. They fed the raw ground beans both dry and moistened and report slightly higher values for the moistened raw beans.

EXPERIMENTAL PROCEDURE

In the preparation of all the beans used in this study they were first sorted so that only perfect average-size specimens were used. Twenty-gram portions were soaked overnight (15 to 17 hours) in approximately 110 c.c. of distilled water. The beans were baked in pyrex glass dishes, at the simmering temperature, 90 to 95°C.(194 to 203°F.). During the first three and a half hours of baking the dishes were covered with watch glasses; after this length of time the covers were removed so that evaporation of the liquid might take place. When most of the liquid had evaporated, the covers were again replaced and the beans cooked until tender. It was intended that the amount of water used should be such that litttle or none would remain after the beans were cooked. When a small amount was left, the beans were mashed with the water, and weighed samples of the

mashed material were used so that none of the liquid was lost. The baking period was approximately nine hours, at which time the beans were tender but still light cream in color and not browned. The beans were cooled at room temperature and stored in the freezing unit of the refrigerator. The raw beans were ground to a powder and weighed portions were mixed with some of the basal diet. As not all of this study was carried out in the same year, two lots of beans were used. The responses of these groups, however, were comparable and are averaged in the results.

The rat-growth method of determination of vitamin B_1 was used in this study. The young white rats were taken from the stock colony when they were 28 days old and weighed 40 to 50 grams. All of the animals were housed individually in metal cages with raised screen bottoms of one-half inch mesh to prevent access to feces. They had access at all times to distilled water and the basal diet. This diet, which consisted of

TABLE 1

Summary of Vitamin B_1 Content of Dried Beans

Food	Units per 100 grams		Investigator
	B-absorbate	Thiamin hydrochloride	
Navy (dried)	......	250	Aughey and Daniel
Navy (dried)	......	125	Booher and Hartzler
Robust (dried	200-210	240	Kelly, Dietrich, and Porter
Michelite (dried)	180	200	Kelly, Dietrich, and Porter
Michelite (dried)	......	170	Kelly and Porter
Navy (commercial canned)	40 [1]	......	McCollum
Navy (boiled)	......	240	Aughey and Daniel
Michelite (boiled in soaking water, 50 min.)	......	300	Kelly and Porter
Michelite (baked in soaking water at 160°C. for 2 hrs.. 45 min.)	......	230	Kelly and Porter

[1] With the exception of this figure all vitamin B_1 units are per 100 grams dry weight.

18 per cent casein, eight per cent butterfat, two per cent cod liver oil, four per cent Osborne and Mendel salt mixture, 15 per cent autoclaved yeast, 28 per cent cornstarch, and 25 per cent sugar, was believed adequate in all respects except for vitamn B_1 for the test period of five weeks. The fore-period in which the test animals were depleted of their vitamin B_1 stores took approximately two to three and a half weeks. During this period the animals were fed the basal diet *ad libitum* and no other food. At the end of the "depletion period" the test food was administered in addition to the diet at will. The food to be tested was fed six days a week throughout the five-week experimental period.

One animal from each litter was used for a negative and usually one for a positive control, and an even distribution of sexes was made in these groups. Positive-control animals were fed B-absorbate in addition to the basal diet. Twelve animals served as negative controls and nine as positive controls.

The remaining animals in the litters were equally distributed and fed two levels of baked beans and one level of dry, raw, ground beans. A

total of 39 animals received the bean supplements. Weekly weights and weekly food-consumption records were kept on all animals. The baked beans were fed in amounts equivalent to .2 and .3 gram of the original dried beans. The average weight of a sample of baked beans equivalent to .2 and .3 gram of dried beans was .636 and 1.025 grams, respectively. As the positive controls were fed B-adsorbate, the amount of beans which would provide one International unit of vitamin B_1 (1930) standard) was determined as well as the weight of beans containing one Sherman unit.

DISCUSSION OF RESULTS

The responses of negative- and positive-control rats are recorded (Table 2); after a depletion period of approximately two to three and a half weeks, the average weight was 65.5 and 78.3 grams for the negative and positive controls, respectively. Negative-control animals lost an average of 1.3 grams per week and survived about 28 days of the experimental period. The positive controls, receivinfi one-half and one International unit doses, made an average gain of 3.3 and 10.9 grams per week, respectively.

The responses of animals fed the bean supplements are given (Table 2). The average length of the depletion periods for these animals was 18 to 23 days. When the bean supplements were begun, the average weight of the animals was 77.5, 83, and 67.7 grams for the .2 and .3 gram equivalents and the .2 gram dry beans. The average weekly food consumption was 30, 33, and 29 grams for the three groups, respectively, during the test period.

As indicated (Table 2) the average gains of the rats on the .2 and .3 gram equivalent and positive control was 3.2, 3.6, and 3.3 grams, respectively. Therefore, it was concluded that an amount of baked beans equivalent to .2 gram of dried navy beans contains approximately on Sherman unit or one-half an International unit (1930 standard). The actual difference, however in mean gains for the two levels is small, amounting to .4 gram. This difference is less than its probable error, and thus does not attain statistical significance (Table 2). The average growth curves of all animals are shown (Fig. 1).

The above results agree with Waterman and Ammerman's (1935) comparison of units of vitamin B_1. They state that one International unit (1930 standard) is equal to two Sherman units. This study confirms their observation.

The vitamin B_1 value of the baked beans was found to be considerably higher than that of the original raw beans. One hundred grams of baked beans calculated on a dry basis contains 250 I. U., whereas, the gains made by the rats on the dry beans were so low, it was not possible to determine their value in units.

The animals receiving the baked beans as a supplement to their vitamin B_1 deficient diet made greater growth responses than those on the dry raw beans. This study, therefore, confirms the study of Kelly and Porter (1940) in which they conclude that cooking processes rendered the vitamin B_1 content of the beans more readily available to animals.

TABLE 2

Food Consumption and Growth Responses of All Experimental Animals

Daily supplement	Quantity fed daily	Number of rats	Average length of depletion period	Average weight after depletion period	Average weekly basal food consumption	Average weekly gains in experimental period	Probable error	Difference	Ratio of difference P. E.
			days	*gm.*	*gm.*	*gm.*			
Negative controls	0	12	19	65.6	27	—1.7	.....	.....	....
B-adsorbate	5 mg.	4	15	85.0	31	3.3	.....	.....	....
	10 mg.	5	22	71.6	46	10.9	.....	.....	....
Baked navy beans	.2 gm. equivalent	17	19	77.5	30	3.2	.63	.4 ±.73	.5
Baked navy beans	.3 gm. equivalent	14	18	83.0	33	3.6	.37	.....	....
Dry navy beans	.2 gm.	7	23	67.7	29	0.2	.....	.....	....

In comparing the vitamin B_1 content of baked beans with that reported by Kelly and Porter it was found that the beans are of approximately the same potency. This is interesting to note for the length of cooking time and temperatures were not alike. The baking temperature and time of cooking for this study was 90 to 95°C.(194 to 203°F.) for nine hours as compared with 160°C.(320°F.) for two hours and 45 minutes by Kelly and Porter. The latter workers also found that boiled beans were slightly higher in vitamin B_1 than the baked; the higher temperature and longer

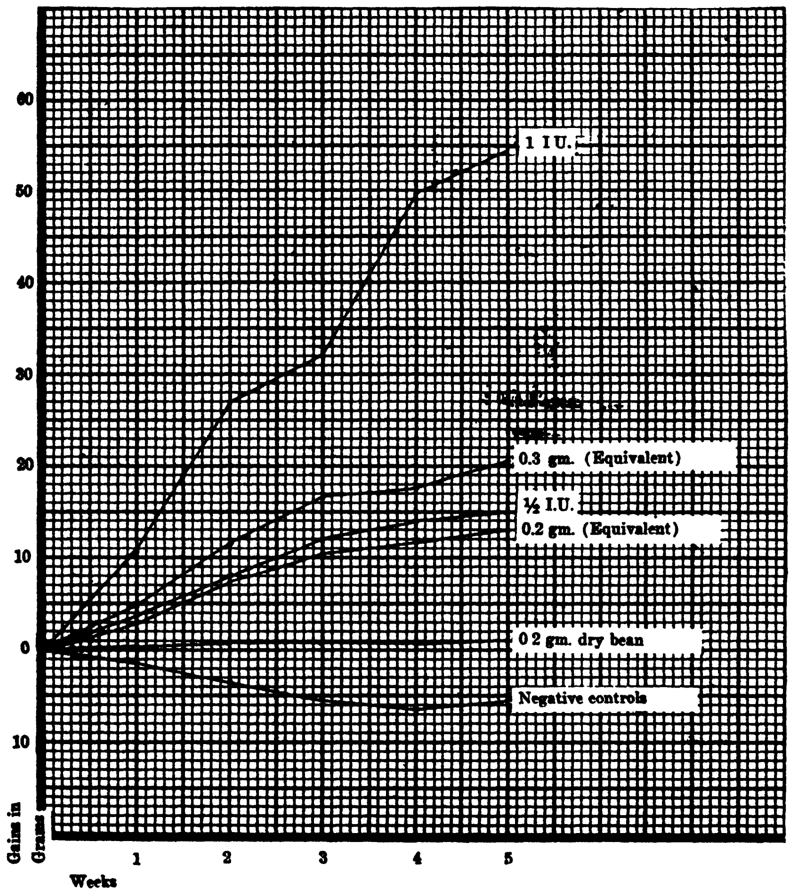

FIG. 1. Average growth curves.

cooking period used for the baking very likely caused destruction of the vitamin. However, the vitamin B_1 content of the boiled beans as reported by Aughey and Daniel is the same as that of the baked beans of Kelly and Porter, and also approximately the same potency as the beans of this study which were cooked at temperatures five to 10 degrees lower and three to six times as long. In commercial canning, where still higher temperatures and pressure are used, the vitamin B_1 value of the beans is considerably lower than the baked beans of these studies, as would be expected.

Of interest to dietitians would be the amount of vitamin B_1 in 100 calories, 100 grams, and a one-ounce portion which are summarized (Table 3).

TABLE 3

Vitamin B_1 Content of Slow-Baked Navy Beans

Beans	Calories	Weight	Sherman units	International units
Baked as fed[1]	100	92.2 gm.	145	72
		100.0 gm.	157	79
		1.0 oz.	45	23
Baked, calculated on a dry basis	100	29.0 gm.[2]	145	72
		100.0 gm.	500	250
		1.0 oz.	142	71

[1] .636 gm. of baked beans are equivalent to .2 gm. dry navy beans. [2] Rose (1929).

SUMMARY

Small white navy beans *(Phaseolus vulgaris)*, called Michigan beans in the Chicago market, were baked according to standard procedures until tender. The vitamin B_1 content of these beans was determined by the rat-growth method with the following results:

Baked navy beans equivalent to .2 gm. of dried navy beans contain approximately one Sherman unit or one-half an International unit (1930 standard). The baked beans weighed approximately three times their original dry weight.

One hundred calories of baked beans contain 145 Sherman units or 72 International units (1930 standard).

One hundred grams of baked navy beans contain 157 Sherman units or 79 International units (1930 standard).

One ounce of baked navy beans contains 45 Sherman units or 23 International units (1930 standard).

This study confirms Waterman and Ammerman's (1935) observation that one International unit (1930 standard) is equal to two Sherman units of vitamin B_1.

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INFLUENCE OF COOKING PROCEDURE UPON RETENTION OF VITAMINS AND MINERALS IN VEGETABLES[1]

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The newer developments in the field of nutrition have indicated that some of the most important elements in our diets, invisible and undetectible by the senses of taste and smell, are lost during the cooking of foods. These are the vitamins and the essential minerals. Prior to these newer developments, the primary function of cooking was to prepare foods in an edible form. In order to preserve the nutritive value of foods, the cook must now look to the nutritionist to ascertain what effects the various operations in the preparation and cooking of foods may have upon the retention of the vitamins and minerals and how these procedures can be so modified as to retain these essential nutrients to the maximum without sacrifice of palatability and appearance.

Most of the studies in this field have been concerned solely with vitamin C losses. This was due to the availability of a chemical method for ascorbic acid analysis while the laborious, costly, and less precise biological procedures were required for the assay of the other vitamins. With the advent of rapid and inexpensive chemical and microbiological test methods, attention was directed toward evaluating the fate of nutrients other than ascorbic acid in cooked foods. These studies, reviewed by Kohman (1942), were confined to a limited number of factors in one or two different types of foods.

The present study was undertaken to determine the difference in degree of retention of vitamins and minerals as affected by variations in culinary methods. Analyses were conducted for the carotene, thiamin, ascorbic acid, riboflavin, nicotinic acid, iron, calcium, and phosphorus contents of four representative vegetables before and after cooking.

The typical vegetables selected were peas, potatoes, carrots, and broccoli representing, respectively, legumes, tubers, roots, and in the last case, leaves, stems, and flowers. Purchases were made in the open market of the freshest vegetables obtainable during the midsummer season. The cooking experiments were conducted on the same mornings that the purchases were made.

Two cooking methods were used, one concerned simply with the preparation of the vegetables in edible form, the other based on a procedure designed also to favor retention of the essential nutrients without sacrifice of palatability. For convenience, the first will be referred to in the present study as the "old-fashioned" procedure and the other as the "new-improved" method of cooking. In the case of the old-fashioned method large proportions of water were used to show the results obtained when no care is observed in the direction of preserving the nutritive elements, as in the

[1] This study is one of a series, sponsored by the Westinghouse Electric and Manufacturing Company, Mansfield, Ohio, as part of its Health for Victory Club program.

days when their existence and importance were not recognized. The vegetables were added to cold, distilled water, brought to a boil, and allowed to simmer until sufficiently cooked. The lid of the cooking pan was kept slightly open to allow the free escape of steam. In the new-improved method of cooking, minimal quantities of water were added to the vegetables and the lid closed flushed with the pan, steam escaping only through the vent in the lid. Steaming was continued until the vegetables were cooked sufficiently to serve. In both methods of cooking, new aluminum pans were used and at no time were the contents stirred.

The details of the cooking procedures used in preparation of each of the vegetables are presented (Table 1). In each case one pound of the vegetables was taken with varying proportions of water depending upon which method of cooking was followed. The net weight of the vegetables after cooking is shown as well as the volume of water drained off. The relatively large differences in evaporation loss (volume of water added minus drainage) in the two methods of cooking obviously cannot be attributed to the slight variations in cooking time but rather to differences in condensing surface and venting of steam.

TABLE 1

Details in Cooking of Vegetables

Vegetable	Method of cooking	Weight taken	Volume of water	Cooking time "High"	Cooking time Simmer	Net weight cooked	Volume of drainage
		gm.	*c.c.*	*min.*	*min.*	*gm.*	*c.c.*
Peas	New-improved	454	15	5	15	434	5
	Old-fashioned	454	360	4	20	453	51
Carrots	New-improved	454	15	5	25	421	31
	Old-fashioned	454	795	6	24	421	310
Potatoes	New-improved	454	60	5	20	464	28
	Old-fashioned	454	454	5	25	491	250
Broccoli	New-improved	454	60	6	24	438	42
	Old-fashioned	454	1,600	5	25	468	613

Immediately after cooking, the vegetables were transferred under an atmosphere of carbon dioxide into a Waring Blendor [2] and a measured quantity of water added. The samples were then homogenized under an atmosphere of carbon dioxide, a uniform period of time being allowed for each vegetable. The homogenized samples were then transferred to bottles and immediately analyzed for those vitamins which might have been affected by standing. Samples were also stored under dry ice pending analyses for those elements not appreciably affected by storage. In the case of the vitamin C analyses of the raw vegetables, aliquots were homogenized with metaphosphoric-acetic acid solution rather than water. Without this precaution, losses of vitamin C may occur because of oxidases liberated during the rupture of the cells; in the cooked vegetables this homogenization with acid is not necessary since the enzymes are inactivated by heat, according to King and Tressler (1940). Use of water or

[2] Obtained from the Waring Corporation, New York City.

acid in the homogenization of the cooked samples yielded the same ascorbic acid values for the final acid extracts.

METHODS OF ANALYSIS

The vegetables were analyzed for carotene (provitamin A), thiamin, ascorbic acid, riboflavin, nicotinic acid, iron, calcium, and phosphorus. The vitamin assays were conducted entirely by chemical and microbiological procedures since these afford a satisfactory basis for estimation of relative vitamin content and have the advantage of yielding results from a single sample within a relatively short time.

Carotene was determined by the method of Fraps, Meinke, and Kemmerer (1941) which involves colorimetric estimation of the pigment selectively extracted by solvents. Thiamin was estimated by the thiochrome procedure of Hennessy (1941). The titrimetric method of Bessey and King (1933) and Musulin and King (1936), involving the use of the dye 2-6 dichlorophenolindophenol, was employed for the determination of ascorbic acid.[3] The microbiological procedure of Snell and Strong (1939) was used for the estimation of riboflavin. Nicotinic acid was determined by the colorimetric method of Melnick, Oser, and Siegel (1941), involving reaction of the vitamin with cyanogen bromide and aniline. The minerals, calcium, phosphorus, and iron, were determined by the official methods of analysis of the Association of Official Agricultural Chemists (1940). Calcium was estimated by the permanganate titration of the oxalate; phosphorus, gravimetrically as magnesium pyrophosphate; and iron, by colorimetric evaluation of the thiocyanate.

Some of the vitamin analyses were not carried out in the case of potatoes and carrots since the nutrients were not regarded as present initially in significant amounts.

DISCUSSION OF RESULTS

The analytical data for the four vegetables in the raw state and ready to serve after cooking by both methods are presented (Tables 2 to 5). In calculating the percentage losses both the concentration of the nutrients in each vegetable and the total yield of vegetables from one pound of raw material were taken into account. In a few instances the total yield of a given vitamin in a cooked product appeared to be greater than that initially present in the raw state. This apparent discrepancy is attributed to three factors: first, limitation in the precision of the analytical methods employed; second, the greater difficulty experienced in extracting the vitamins for analysis from the vegetables in the raw state; and third, the probable action of destructive enzymes in raw vegetables released during homogenization, Fraps, Meinke, and Kemmerer (1941); King and Tressler (1940). These enzymes are, of course, inactivated when vegetables are

[3] This visual titrimetric method is satisfactory for testing freshly picked vegetables since the materials are relatively free from interfering nonspecific reducing substances. In the preparation of the samples for ascorbic acid analyses, homogenization of the raw vegetables with metaphosphoric acid or of the cooked samples with water, both under carbon dioxide, inhibited the formation of dehydroascorbic acid. The ascorbic acid oxidases, naturally present in these vegetables, are inactivated in the former case by the metaphosphoric acid medium and in the latter by the prompt cooking of the samples, Hochberg, Melnick, and Oser (1942).

TABLE 2

Influence of Cooking Procedure Upon Loss of Vitamins and Minerals in Peas

Nutrient	Raw peas	Cooked Peas		Change in total content of nutrient[1]			Loss of nutrient in cooking	
		New-improved	Old-fashioned	Initial	After new-improved cooking	After old-fashioned cooking	New-improved	Old-fashioned
	microgm./gm.	*microgm./gm.*	*microgm./gm.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*
Carotene	7.65	7.2	6.3	3.47	3.12	2.85	10	18
Thiamin	4.8	4.7	2.6	2.18	2.04	1.18	6	46
Ascorbic acid	321	287	218	146	125	99	14	32
Riboflavin	2.58	2.51	1.79	1.17	1.09	0.81	7	31
Nicotinic acid	17.9	18.2	11.5	8.13	7.90	5.21	3	36
Iron	16	16.5	16.7	7.26	7.16	7.57	1	0
	mg./gm.	*mg./gm.*	*mg./gm.*	*gm.*	*gm.*	*gm.*		
Calcium	0.22	0.23	0.21	0.100	0.100	0.095	0	5
Phosphorus	1.14	1.14	1.04	0.518	0.495	0.471	4	9

[1] The weight of each sample before and after cooking is given in Table 1.

TABLE 3

Influence of Cooking Procedure Upon Loss of Vitamins and Minerals in Carrots

Nutrient	Raw carrots	Cooked carrots		Change in total content of nutrient[1]			Loss of nutrient in cooking	
		New-improved	Old-fashioned	Initial	After new-improved cooking	After old-fashioned cooking	New-improved	Old-fashioned
	microgm./gm.	*microgm./gm.*	*microgm./gm.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*
Carotene	96.8	100.1	97.6	43.9	42.1	41.1	4	6
Nicotinic acid	7.2	7.7	5.5	3.27	3.24	2.32	1	29
Iron	3.9	4.05	3.6	1.77	1.71	1.52	3	14
	mg./gm.	*mg./gm.*	*mg./gm.*	*gm.*	*gm.*	*gm.*		
Calcium	0.41	0.44	0.41	0.41	0.185	0.173	1	7
Phosphorus	0.18	0.21	0.18	0.18	0.088	0.076	0	7

[1] The weight of each sample before and after cooking is given in Table 1.

TABLE 4

Influence of Cooking Procedure Upon Loss of Vitamins and Minerals in Potatoes

Nutrient	Raw potatoes	Cooked potatoes		Change in total content of nutrient[1]			Loss of nutrient in cooking	
		New-improved	Old-fashioned	Initial	After new-improved cooking	After old-fashioned cooking	New-improved	Old-fashioned
	microgm./gm.	*microgm./gm.*	*microgm./gm.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*
Thiamin	1.7	1.6	1.1	0.77	0.74	0.54	4	30
Ascorbic acid	270	260	220	123	120	108	2	12
Riboflavin	0.67	0.64	0.34	0.31	0.30	0.17	3	45
Nicotinic acid	8.6	9.0	5.9	3.90	4.18	2.90	0	26
Iron	6.0	5.4	4.6	2.72	2.51	2.26	8	17
	mg./gm.	*mg./gm.*	*mg./gm.*	*gm.*	*gm.*	*gm.*		
Calcium	0.084	0.07	0.07	0.038	0.033	0.034	13	11
Phosphorus	0.47	0.47	0.33	0.213	0.218	0.162	0	24

[1] The weight of each sample before and after cooking is given in Table 1.

TABLE 5

Influence of Cooking Procedure Upon Loss of Vitamins and Minerals in Broccoli

Nutrient	Raw broccoli	Cooked broccoli		Change in total content of nutrient[1]			Loss of nutrient in cooking	
		New-improved	Old-fashioned	Initial	After new-improved cooking	After old-fashioned cooking	New-improved	Old-fashioned
	microgm./gm.	*microgm./gm.*	*microgm./gm.*	*mg.*	*mg.*	*mg.*	*pct.*	*pct.*
Carotene	8.6	9.3	9.2	3.90	4.07	4.31	0	0
Thiamin	0.86	0.85	0.47	0.39	0.37	0.22	5	44
Ascorbic acid	950	660	430	431	289	201	33	53
Riboflavin	3.20	2.43	1.95	1.45	1.06	0.91	27	37
Nicotinic acid	9.1	8.3	3.2	4.13	3.64	1.50	12	64
Iron	8.4	8.4	8.4	3.81	3.68	3.93	3	0
	mg./gm.	*mg./gm.*	*mg./gm.*	*gm.*	*gm.*	*gm.*		
Calcium	0.68	0.57	0.47	0.309	0.25	0.220	19	29
Phosphorus	0.54	0.48	0.38	0.245	0.21	0.178	14	27

[1] The weight of each sample before and after cooking is given in Table 1.

cooked. The conclusion has been drawn in these instances that the vitamin or mineral in question was completely retained.

In the case of peas cooked by the new-improved method most of the vitamins, from 86 to 97 per cent, and practically all of the minerals were retained. The losses of vitamins following the old-fashioned method of cooking were consistently and significantly greater. The mineral retention, however, was practically as good. The greatest losses were observed in the case of the water-soluble vitamins, thiamin, ascorbic acid, riboflavin, and nicotinic acid.

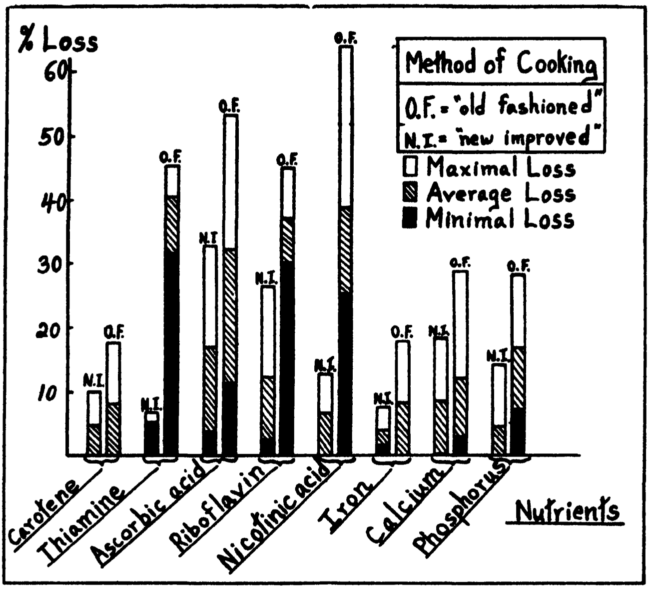

FIG. 1. Average and range of loss of vitamins and minerals in vegetables subjected to the "old-fashioned" and "new-improved" methods of cooking.

In the case of carrots and potatoes the same differences in retention of vitamins as a result of the two methods of cooking were noted. In addition there were significant losses of minerals following the old-fashioned method of cooking the vegetables.

Broccoli, in contrast with the other three vegetables, showed significant losses of the water-soluble vitamins when cooked by the new-improved method. However, tremendous losses of the water-soluble vitamins were noted upon cooking by the old-fashioned method, varying from 37 per cent (riboflavin) to 64 per cent (nicotinic acid). Carotene alone was completely retained. Significant differences in the retention of calcium and phosphorus were observed in the broccoli cooked by the two methods, the losses following the old-fashioned method being almost twice as great as those noted in the product cooked by the new-improved method.

CONCLUSIONS

The range and average loss of each of the nutrients for the two methods of cooking are graphically represented (Fig. 1). The results show con-

clusively that cooking in large volumes of water, characteristic of old-fashioned methods, results in substantial loss of the minerals and more particularly of the water-soluble vitamins of vegetables. These losses are markedly reduced by the improved method of cooking involving the use of minimal proportions of water with provision for recondensation of the steam. Except for a few isolated cases, particularly with respect to broccoli where the losses are, in general, somewhat greater regardless of the method of cooking, it may be said that more than 90 per cent of the vitamins and minerals are retained following the improved method of cooking.

The results obtained in the present study are in good agreement with those recently reported by Olliver (1941); Brinkman, Halliday, Hinman, and Hamner (1942); and Woodruff and Scoular (1942) on the effect of various cooking procedures on the retention of those nutrients studied in common in the investigations.

SUMMARY

Four vegetables—peas, potatoes, carrots, and broccoli, representing, respectively, legumes, tubers, roots, and in the last case leaves, stems, and flowers—were subjected to two methods of cooking: one the old-fashioned procedure representative of methods in which no attempt is made to retain the essential vitamins and minerals; the other, a new-improved method designed to retain these nutrients to a maximal degree. Analyses were made of the raw and cooked products for carotene, thiamin, ascorbic acid, riboflavin, nicotinic acid, iron, calcium, and phosphorus. The large losses of vitamins noted following the old-fashioned method of cooking, averaging 31 per cent, were greatly reduced by the new-improved procedure so that on the average more than 90 per cent of these nutrients were retained. The losses of minerals in either case were not large, averaging 12 and five per cent, respectively.

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FURTHER STUDIES ON THE THIAMIN VALUES OF FROZEN PEAS [1]

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Few studies have been reported on thiamin content of frozen peas. Rose and Phipard (1937) found no difference between the vitamin B_1 values of raw fresh and frozen peas of one year, but the frozen peas from the commercial packs of two different years varied greatly. Fellers, Esselen, and Fitzgerald (1940) found a loss of three per cent of the vitamin B_1 in the freezing of peas. Fincke (1939) observed an inverse relationship between the time and temperature of blanching and the thiamin content of peas, but the difference between those blanched at the lowest temperature for the shortest time and those blanched at the highest temperature for the longest time was barely significant. A wide varietal difference in frozen peas was, however, found. Richardson and Mayfield (1940) reported on the vitamin B_1 content of raw frozen peas before and after cooking but did not compare them with fresh, unfrozen peas.

In the previous publication from this laboratory (1939), no comparison between the thiamin values of fresh and frozen peas was made because the method available at the time was the rat-growth method, and it was impossible to keep fresh peas of one lot throughout an entire feeding period. The present study is a continuation of that work. Peas of different varieties, after various treatments, were studied. Thiamin values were measured of peas in the raw fresh state, cooked fresh, uncooked frozen and cooked frozen, brine-packed and dry-packed, and peas grown in different parts of the state in different years.

EXPERIMENTAL PROCEDURE

The peas used in this study were grown by the Horticulture Department of Oregon State College and frozen by the Food Industries Department. The assistance of these two departments is sincerely acknowledged.

The peas, except the Thomas Laxton variety, were all blanched in a similar manner, packed in cartons, and placed in a room kept at —18°C. (—0°F.) or lower. The Thomas Laxton peas were blanched in the manner recommended for home preparation by Diehl, Wiegand, and Berry (1939), packed in cartons, and placed in a frozen storage locker kept at —18 to —16°C.(—0 to 3°F.).

The thiamin values were determined by the rat-curative method as described in the U. S. Pharmacopoeia (1940). Each animal was given a measured dose of thiamin and the length of the cure noted; then a weighed amount of peas was fed and the length of cure again noted. The thiamin

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content of the peas was calculated from the curve of Fincke and Little (1941) which had been plotted from the responses to graded amounts of thiamin of a large number of animals from this laboratory, which was rechecked by giving known doses of thiamin to littermate controls of the experimental animals. Usually two different levels of peas were fed with eight or nine animals on a level. The results from these two levels agreed well. During 1938-39 peas were tested both by the rat-growth method and the rat-curative method. Alderman peas tested by growth method showed a thiamin value of 3.8 micrograms per gram and by the curative method, 4.0 micrograms per gram. As the results from the two methods agreed well, they were averaged for the final report. In weighing the test dose of peas, 40 to 50 grams were finely chopped while in the frozen state and the amount to be fed (two to three grams) was taken from the well-mixed lot.

When peas were cooked, 40 to 50 grams were weighed in the frozen state, placed into a beaker containing 20 ml. of rapidly boiling water, covered, and cooked five minutes for fresh or three minutes for frozen peas after the water returned to the boil. Previous tests had been carried out to determine the time of cooking until just done. They were then drained for 30 seconds, weighed, chopped, and well mixed. Peas were all fed on the basis of uncooked weights, the amounts of cooked peas which corresponded to given amounts of uncooked peas being weighed. Thiamin values were all reported on the basis of uncooked weight. Brine-packed peas were thawed and cooked in the same solution in which they had been frozen.

RESULTS AND DISCUSSION

Effect of Freezing: The thiamin values of fresh and frozen peas (Table 1) show that of the three varieties analyzed in both the fresh and frozen state, while the thiamin content of the frozen peas was in each case slightly lower than that of the fresh, in no case was the difference significant. The avearge of the three varieties when fresh was 3.17 micrograms per gram of peas and when frozen, 2.9 micrograms per gram, an apparent loss of about 8.5 per cent. The total moisture contents of the fresh and frozen were practically the same, i.e., 79.3 per cent for the fresh and 79.1 per cent for the frozen. This loss in thiamin values compares closely with the findings of Fellers and his coworkers (1940) who reported a loss of three per cent in freezing.

Effect of Cooking: Fresh Thomas Laxton peas had a thiamin content of 3.1 micrograms per gram when uncooked and, on the basis of uncooked weight, 3.0 micrograms per gram after cooking, as shown (Table 1). When frozen, they contained 3.0 micrograms per gram before cooking and 2.8 micrograms per gram after cooking. This loss is not significant. Some peas which had been packed in brine contained 3.7 micrograms per gram before cooking and 3.6 micrograms per gram after cooking. Duplicate lots which had been dry-packed contained 3.9 micrograms per gram before and 3.3 micrograms per gram after cooking. This does not represent a significant difference between uncooked and cooked or between brine-packed and dry-packed peas. These peas were all cooked in a small amount of water for a short time. Although the quantities of peas cooked were small, the rela-

tive amounts of peas and liquid were about the same as might have been used in cooking enough for four servings, and the time of cooking was about the same. However, this small amount of peas returned to the boil more quickly than would the amount usually cooked in a home.

This small loss in cooking (about 10 per cent), small enough to be within the limits of experimental error, may be compared with the findings of Rose and Phipard (1937) that peas cooked for 15 minutes, during which time practically all the water disappeared, lost 26 per cent of their thiamin values. Aughey and Daniel (1940) observed that peas which were simmered for 12 minutes in half their volume of water lost 11 per cent of the thiamin in the cooking liquid while an additional nine per cent was destroyed. Baker and Wright (1938) found that raw fresh peas contained 1.6 to 2.8 I.U. per gram, while cooked fresh peas of the same lot contained .8 to 1.2 I.U. per gram. Cooking method was not described. Richardson

TABLE 1

Thiamin Values of Fresh and Frozen Peas, Cooked and Uncooked

Variety	Fresh peas		Frozen peas	
	Number of animals	Thiamin (micrograms per gram)	Number of animals	Thiamin (micrograms per gram)
Onward	7	3.1±.35 [1]	16	2.6±.34 [1]
Asgrow Glacier	14	3.3±.32	18	3.1±.31
Thomas Laxton, raw	9	3.1±.16	18	3.0±.17
Thomas Laxton, cooked	9	3.0±.14	9	2.8±.32
Dry-packed, uncooked	....	....	19	3.9±.16
Dry-packed, cooked	....	....	9	3.3±.32
Brine-packed, uncooked	....	....	24	3.7±.17
Brine-packed, cooked	....	....	9	3.6±.37

[1] Standard error of the mean, $S_M = \sqrt{\frac{\Sigma}{N(N-1)}}$

and Mayfield (1940) reported that raw green peas (Laxtonian) contained 1.02 I.U. thiamin per gram, and after boiling 30 minutes, .85 I.U. per gram, a loss of 16.7 per cent in cooking. The peas used in this study needed only a short cooking time, which may account for the smaller loss than others have found.

Effect of Environmental Factors: In order to study the effect of environment on the thiamin content of peas, the same variety of pea was grown in one year in three different parts of the state, i.e., Corvallis, Astoria, and Pendleton, and these were frozen after the same kind of processing. In addition, the same variety was raised in the same place two different years. Astoria is in the narrow coastal plain where the air contains much moisture and the temperature is moderately cool and well suited to the development of peas. Corvallis is in the Willamette valley between the low coast range and the high Cascades, the climate being dry and variable as to temperature in the pea-growing season; while Pendleton lies east of the Cascades in the irrigated portion of the upper Columbia valley. The temperatures in Pendleton are in general higher than in either of the other two sections, and the altitude is higher. All three sections of the state raise peas commercially as an important crop.

Tall Alderman peas grown in Pendleton in 1938 contained 2.9 micrograms per gram while those grown in the same place in 1939 contained 4.5 micrograms per gram (Table 2). This represents a significant difference; but the average of the two values, 3.7 micrograms per gram, is close to the value of 3.9 micrograms per gram for those grown in Corvallis and Astoria. No significant difference was observed in the Improved Gradus peas from different places, those grown in Corvallis containing 4.5 micrograms per gram and those from Pendleton, 4.7.

TABLE 2

Effect of Year and Locality on Thiamin Values of Frozen Peas

Variety	Where grown	Year	Number of animals	Thiamin (micrograms per gram)
Alderman	Corvallis	1938	8	3.9±.78 [1]
Alderman	Pendleton	1938	25	2.9±.20
Alderman	Pendleton	1939	8	4.5±.54
Alderman	Astoria	1939	15	3.9±.46
Improved Gradus	Corvallis	1938	9	4.5±.14
Improved Gradus	Pendleton	1938	12	4.7±.45
Extra Early Gradus	Oregon	1938	23	2.8±.22
Extra Early Gradus	Washington	1936	17	5.0±.66
Stratagem	Oregon	1939	27	7.3±.39
Stratagem	Washington	1936	17	5.2±.38
Asgrow 40	Oregon	1938	25	3.8±.22
Asgrow 40	Washington	1936	17	7.4±.50

[1] $S_M = \sqrt{\frac{\Sigma x^2}{N(N-1)}}$

TABLE 3

Average Thiamin Values of Peas of Different Varieties

More than 6 micrograms per gram		Less than 4 micrograms per gram	
World Record	7.1	Extra Early Gradus	3.9
Lord Chancellor	7.0	Tall Alderman	3.8
Dwarf Alderman	6.7	Glacier Park	3.6
Rogers 95	6.5	Telephone	3.3
Stratagem	6.3	Glacier	3.3
		Asgrow Glacier	3.1
4 to 6 micrograms per gram		Thomas Laxton	3.0
Asgrow 40	5.6	Gradus	2.9
Improved Gradus	4.6	Improved Stratagem	2.7
Confidence	4.3	Onward	2.6
		Laxton's Progress	2.0

In comparing peas grown and frozen in Oregon with the same varieties previously analyzed, which were grown and processed in Washington and previously reported, rather great differences were found. These were not controlled comparisons since the two lots of peas were not grown under the direction of the same person and the source of the seed and processing practices may have been different. The difference between peas of the Extra Early Gradus, Stratagem, and Asgrow 40 varieties grown in Oregon and Washington is shown (Table 2).

Variety: In a previous study (1939) the thiamin values of 10 varieties of peas were reported. Several more varieties have been analyzed for thiamin and the values of all varieties are listed (Table 3). Values obtained from different lots of the same variety grown in different years have been averaged. The thiamin values range from 7.1 micrograms per gram for World Record down to 2.0 micrograms per gram for Laxton's Progress. A half-cup serving of peas weighs about 65 grams. As little thiamin is lost in cooking for a short time, all of these peas would provide significant amounts of thiamin in a day's dietary, while those in the upper part of the list would contribute a large part of the day's standard allowance.

SUMMARY

The thiamin values of fresh and frozen peas, before and after cooking for a short time in a small amount of water, have been determined. Under our experimental conditions no significant differences between fresh and frozen, uncooked and cooked peas were found.

Environment may affect the thiamin values of peas, as those grown in one place in 1938 contained significantly less thiamin than those grown in the same place the following year. On the other hand, certain varieties of peas grown in different parts of the state the same year contained approximately the same amounts of thiamin.

The thiamin values of several varieties of peas were determined, and were found to range from 2.0 micrograms per gram in Laxton's Progress to 7.1 micrograms per gram in World Record.

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A MICROSCOPIC STUDY OF THE PHYSICAL CHANGES IN CARROTS AND POTATOES DURING DEHYDRATION[1]

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The present war has lent impetus to the dehydration of foods, but even prior to the war there was interest in this means of conserving and utilizing perishable surpluses. Among technical studies designed to improve techniques and products, microscopic investigations have a place because they show the alterations in internal structure that are brought about by various processes.

One of the problems that may be encountered in dehydration of certain vegetables is "case hardening." This results when the outer tissues of sliced or diced vegetables dry more rapidly than the inner tissues and seal together during the early stages of dehydration. Chace, Noel, and Pease (1941) have pointed out that the development of a hardened outer case during the earlier stages of dehydration inhibits evaporation from the inner portions of thickly sliced or diced vegetables. They have described methods of controlling the initial rates of evaporation so that the tissues dry more uniformly and evaporation from the inner tissues is more complete. Such control is particularly necessary if the pieces of vegetable are over one-fourth inch thick. The physical changes of the cells and the cell contents, during dehydration, constitute the subjects of the present paper.

Fully grown carrots were used as a tissue "type" for fleshy vegetables low in starch, and white potatoes were used as a "type" for starchy vegetables. A discussion of the anatomy of a member of the carrot family may be found in publications by Hayward (1938) and Winton and Winton (1935). The development of the carrot is discussed in detail by Esau (1940). In brief, the edible portion of the carrot consists of an inner core separated by cambium from an outer cylinder of phloem and parenchyma. Strands of lignified xylem ("X" in Fig. 2) are interspersed with parenchyma in the center of the core. The xylem is in radial rows at the periphery of the core. Upon dehydration, the lignified xylem cells shrink the least and display a minimum of cell-wall collapse. The parenchyma cells, on the other hand, collapse together until they are indistinguishable in completely dried tissue.

All the living, mature parenchyma cells in both core and cylinder are highly vacuolate. Crystalline inclusions are mainly the carotene granules, which are more abundant in the phloem regions than in the core. These occupy only a small percentage of the total volumes of the cells. Consequently, with removal of water from the cells there is not enough bulk of cell contents to limit the amount of shrinking the cells will undergo during dehydration.

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Young carrots about half grown contain variable amounts of starch in the phloem regions. It is present in sufficient quantity in some of the cells to limit their shrinking during dehydration. As the carrot matures this starch is converted into other plant products.

The anatomy of the potato tuber is also discussed by Hayward (1938) and Winton and Winton (1935). The bulk of the tissue is storage parenchyma containing abundant starch. The starch grains range up to 100 μ long and as many as 30 or 40 may be crowded into a single cell. White potatoes average about 80 per cent water content and some of this water is present in the starch grains.

HISTOLOGICAL TECHNIQUE

Preparation of the dehydrated samples for microscopic examination presented a number of difficult problems. The samples could not be softened for sectioning by even partial reconstitution without destroying the effects of dehydration. Later stages of dehydration were virtually impossible to section without severe damage to the microtome knife even when the samples were embedded in celloidin.

A modified celloidin impregnation and embedding method produced the most satisfactory results. Samples were collected at half-hour intervals of drying and immediately placed in screw-cap vials containing absolute alcohol. They were then evacuated in order to remove as much of the trapped air as possible. This treatment caused some distortion of the less shrunken cells, but it did not destroy the general effects of dehydration. The samples were then impregnated with celloidin, as described by Johansen (1940), in a paraffin oven at 55°C.(131°F.). The schedule required at least a week of heat treatment in order to effect an adequate celloidin penetration.

Potato samples approaching complete dehydration were too hard to section without first trimming the celloidin block to expose the material slightly, and then submerging it in glycerine-alcohol for several hours to soften the tissue. Even with this treatment, only fragmentary sections could be obtained.

Some of the sections were stained with fast green in a solution of seven parts xylene to three parts ethyl alcohol, or with safranin in absolute alcohol followed by differentiation in the fast green solution. Aqueous and dilute alcohol solutions of the stains produced partial rehydration effects on the tissues and therefore were not used. After staining, the sections were washed in alcohol and cleared in xylene before mounting in Canada balsam. Photomicrographs were taken at various magnifications, using an orange gelatin filter to produce photographic contrast.

SHRINKAGE DURING CARROT DEHYDRATION

Steam scalding prior to dehydration results in a more uniform evaporation from carrot tissues during the earlier stages of dehydration, but it does not prevent the development of an outer case of excessivley shrunken cells. The cells of the blanched carrot are plasmolyzed and the cytoplasm is denatured. In the living state the cells are turgid and the cytoplasm possesses selective permeability. When raw carrot is dehydrated the outer

cells are first affected by initial water loss and by denaturization of the cytoplasm. The initial evaporation rate tends to lower the wet-bulb temperature (of the carrot) and retards the effects upon the inner tissues.

It is doubtful that "case hardening," as such, takes place in carrot tissues unless young, starch-containing carrots are used and are thickly sliced. With fully grown carrots there appear to be no differences in hardness between blanched and unblanched, dehydrated samples or between the outer cases of these formed in early periods of drying and the completely dried inner tissues.

The parenchyma tissue of the core appears to undergo less severe shrinkage than does the outer cylinder because of the lignified xylem cells which tend to maintain their form and structure and hold the surrounding cells in place. At the periphery of the slices and along the cut, transverse margins of the phloem, the case formed by the initial shrinkage exerts a "pulling" force counter to that produced by internal shrinkage during subsequent dehydration. This is shown by the tendency of the cells underlying the case to be pulled open (Fig. 3). The outer cylinder in diametric view becomes somewhat "T"-shaped as dehydration progresses. The case of more severely shrunken cells is naturally stronger at the corners of the periphery than along the flat, cut surfaces of the slices. Comparable effects take place during the dehydration of cubed or diced carrot.

Photomicrographs are shown of fresh carrot that had been killed in Fleming's solution and prepared by the paraffin method for sectioning (Figs. 1 and 2). The small cells at the top (Fig. 1) are cork and cork cambium (collectively—periderm), which are removed by peeling. The rest of the tissue is pericyclic and phloem parenchyma. The inner portion of the core with the scattered xylem groups and surrounding core parenchyma is shown (Fig. 2).

The degree of cell shrinkage in the inner and outer portions of sliced carrot after two and one-half hours of dehydration may be seen (Fig. 3). A linear unit of 10 average phloem parenchyma cells is from 400 μ to 500 μ in the fresh tissue. The outer layers of severely collapsed cells (Fig. 3) are approximately 300 μ deep and involve from 30 to 50 cell diameters. The irregularities occurring as surface folds are caused by variable tensions produced during dehydration. Some irregularities may arise from xylem strands, belonging to small lateral roots, which pass outward from the core through the phloem and pericycle.

The shrinking produced by dehydration at different temperatures showed no pronounced differences during the earlier stages of drying. The development of the outer case took place more rapidly with increased temperature until the tissue was either completely dehydrated or the shell provided a physical barrier to loss of moisture from the inner tissues. In carrots such a barrier may result from the sealing effect of the pectins in the cell walls, but these pectins vary with the age of the carrot and can constitute only a minor contributing factor. Sections cut from samples dehydrated at temperatures over 100°C.(212°F.) showed a tendency to "cook out" during later stages of dehydration. In other words, when evaporation from the inner tissues was inhibited by the outer case, the trapped moisture vaporized and produced sufficient pressure to rupture

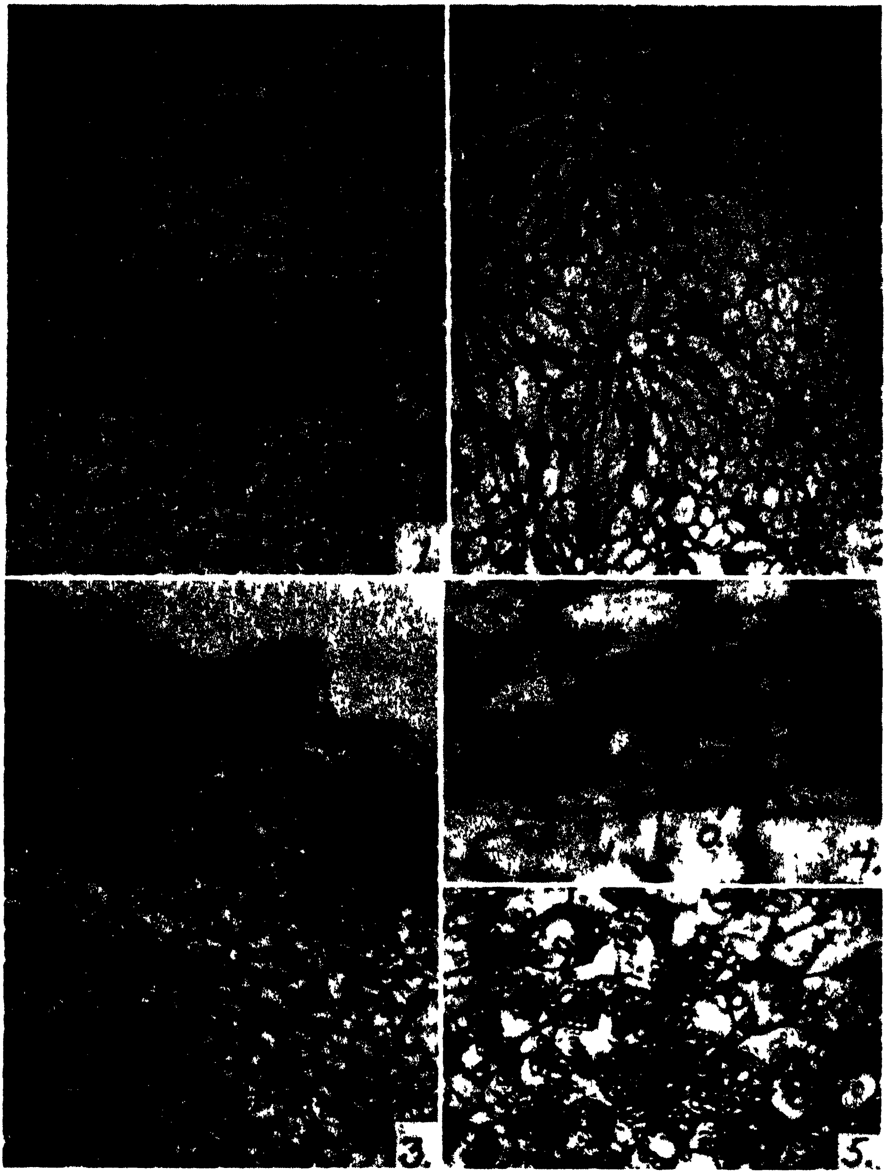

FIG. 1. Section prepared from the outer cylinder of fresh carrot cut in cross section. Od—oil ducts (× 75).

FIG. 2. Section prepared from the core of fresh carrot cut in cross section. X—xylem (× 75).

FIG. 3. Section cut from the phloem region of a carrot slice after two and one-half hours of dehydration (× 100).

FIG. 4. Section cut from reconstituted carrot tissue and stained with Sudan IV to show the distribution of vegetable oil droplets. O—oil droplets, C—carotene granules, W—cell walls (× 200).

FIG. 5. Section cut from fresh potato tissue showing the numerous oval shaped starch grains within the cells (× 75).

the softer, inner tissues. The higher the temperature, the more pronounced this effect. When sections were cut from reconstituted samples that had been "flash" dehydrated at 125°C.(257°F.) or more, the inner tissues were badly macerated. A diametric view of slices of "cooked out" samples showed the centers to be not only full of large and irregular pockets but to have also evidences of caramelization. Such samples reconstitute poorly.

SOME CHARACTERISTICS OF CELL WALLS IN CARROT PARENCHYMA

Depending upon the amount of cell-wall substance deposited and the age of the cells, the walls of phloem parenchyma cells in fresh carrot range from one to two microns in thickness. This was determined by measuring the thickness of adjoining walls between which the middle lamella was clearly visible. When water is removed from the vacuoles and the walls by placing the tissues in absolute alcohol for several minutes, the wall thickness shrinks to approximately one-half micron. This is comparable to the wall shrinkage that results when carrot tissues are prepared by the alcohol-xylene-paraffin technique for sectioning, and the wall shrinkage resulting from commercial dehydration is approximately the same. Measurements of the more severely collapsed cells (Fig. 3) averaged slightly less than one-half micron in thickness. Upon reconstitution of such samples the cellulose walls imbibe water and swell to approximately the thickness of the walls in fresh tissues.

The reconstituted carrot tissue (Fig. 4) shows that the cell-wall structure has remained intact although the cytoplasmic constituents have been altered. This section was stained with Sudan IV, a fat dye, and thus the unstained walls have not registered with sufficient photographic detail to show their true thickness. The vegetable fats (stained with Sudan IV), including true fats, lipo-proteins, and other lipoids, are coalesced into tiny droplets "O" which are dispersed throughout the tissue.

Carrot tissue is known to be high in pectic substances. As previously mentioned, under certain conditions it is conceivable that this pectin may prolong dehydration. This could occur if, during dehydration, the pectins produced a cementing effect in the outer case of more severely shrunken cells. The completely dehydrated cellulose-pectic complex of carrot cell walls creates a much harder product than when, for example, leafy vegetables are dehydrated.

The staining techniques used by Branfoot (1929) and Rawlins (1933) were used to determine the distribution of the pectic materials in carrot tissues. The iron-adsorption method, giving a Prussian Blue reaction, and Ruthenium Red (1:5,000 dilution) both showed the pectins within the cellulose walls and in the middle lamellae. Allen (1901), in his studies on the middle lamella, concluded that it is predominantly calcium pectate. He likewise hypothesized that during growth the cell wall stretching in meristematic cells is possible because the pectic material is in a sol state, whereas in the mature cells the middle lamella pectins are in a gel state.

When sections of fresh carrot, prepared by the paraffin method for sectioning, were placed in cupra-ammonium to dissolve the cellulose and then washed and stained with Ruthenium Red, a pronounced middle lamella region of pectic reaction was obtained. There was also some faint

staining in the cellulose-wall region. Dehydrated samples that had been reconstituted and prepared by the paraffin method for sectioning also showed some pectic material in the middle lamella regions when treated in the same manner. The staining was not so pronounced as in the fresh, prepared sections. This would be expected if some of the water-soluble pectins had been removed by reconstitution. Sections of dehydrated carrot treated without reconstituting showed an indistinguishable mass staining of pectins.

STARCH CHANGES DURING DEHYDRATION OF POTATOES

In contrast to the dehydrated tissues of carrots, there are marked differences between blanched and unblanched potato tissues at different stages of dehydration. Blanching (whether steam or hot water scald) causes swelling and gelation of the starch grains. Provided dehydration is begun immediately after blanching, the starch is in a gel state at the initial periods of drying. Gelation of the starch in raw potato does not occur until later stages of drying if and when the temperature of the potato (wet bulb) has reached approximately 65°C.(149°F.). This was determined by examination of fresh sections placed in laboratory ovens and dried at different temperatures.

Alsberg and Rask (1924) found that the gelation temperature of starches varied according to treatments, horticultural varieties, and different methods of observation. Nyman (1912) established the gelation temperature as that point at which starch grains no longer showed interference crosses when examined under polarized light. The same method was used in this work.

The relation of the gelled starch to the cell walls can be seen in partially reconstituted potato that was blanched before drying (Fig. 7). The dried potato was soaked in cold water, during which time the cellulose walls imbibed water more rapidly than did the gelled starch. The sample was then prepared for sectioning by the paraffin method. In contrast with this, the section of fresh tissue (Fig. 5) shows individual starch grains within the cells. Fully reconstituted potato is shown (Fig. 9). The semifibrous or reticulate appearance of the gelled starch (Figs. 8 and 9) may be due to variations in density as the grains swell or to cytoplasmic substances trapped between the swollen and gelled grains.

Because of the starch content, it does not seem likely that the pectins in potato tissue could be significant in influencing the texture of the dried product. That potato is lower in pectins than is carrot (particularly water-soluble pectins) may be demonstrated by prolonged cooking during which potato macerates more rapidly than does carrot tissue. The pectins dissolve and the cells separate.

COMPARISONS OF STAGES IN DEHYDRATION OF BLANCHED AND UNBLANCHED POTATOES

When raw potato is dehydrated the initial shrinking of the surface cells crowds the starch grains together. If shrinkage is severe, the cell walls may be ruptured and the starch grains may be forced out of the tissue. The structural shrinkage of the cells is obscured by the starch, but

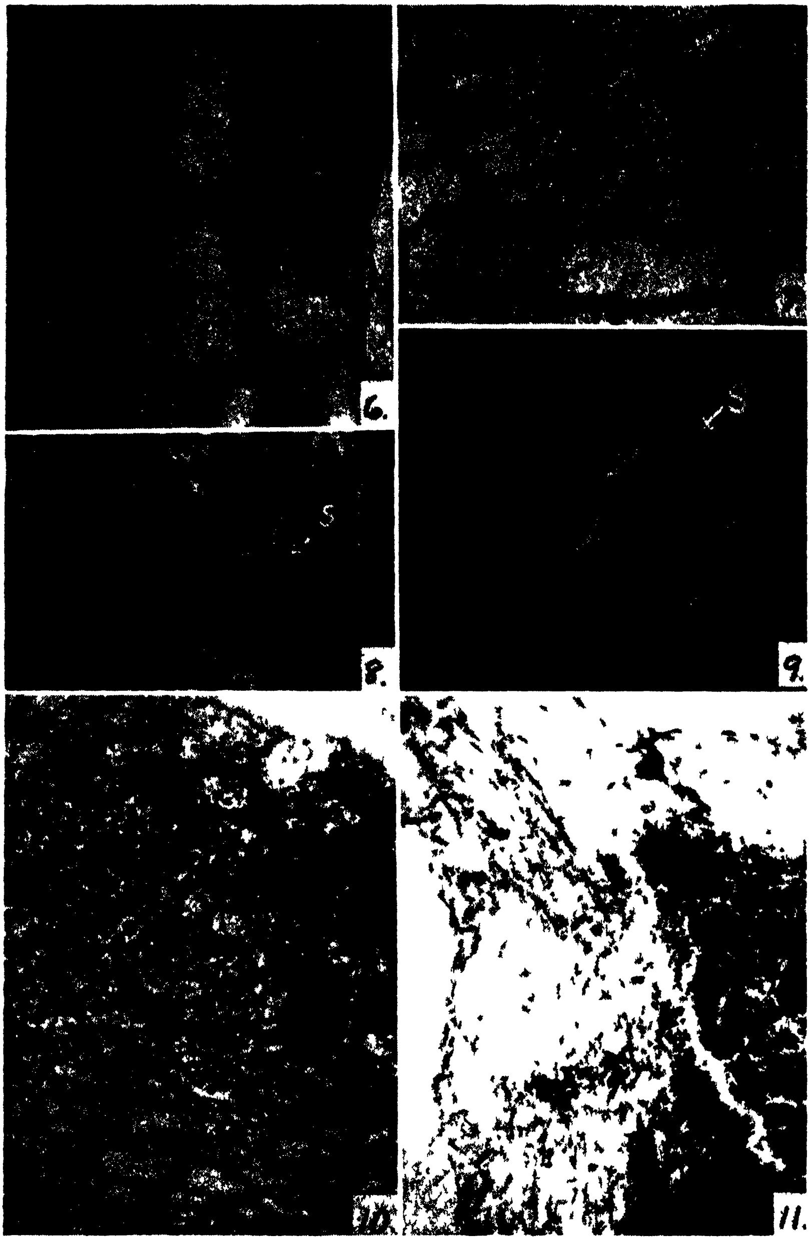

FIG 6 Cell of fresh potato stained with Ruthenium Red. ML—middle lamella. Light shadows are caused by starch grains out of focus (X 650).

FIG 7 Cells of blanched, completely dehydrated potato (X 650).

FIG. 8. Section cut from blanched, dehydrated potato after partial reconstitution. S—gelled starch, W—cell wall (X 200).

FIG. 9. Section cut from blanched, dehydrated potato after complete reconstitution. S—gelled starch (X200).

FIG. 10. Section cut from raw potato after two and one half hours of dehydration; showing gelled starch (S) in the inner tissues and intact starch grains in the outer layers of tissue (X 100).

FIG. 11. Section cut from blanched potato cube after two hours of dehydration showing the development of the outer case (clear cells) of severely shrunken cells (X 75).

the crowding effect may be seen (Fig. 10). During dehydration, gelation of the starch in unblanched samples does not occur until the wet-bulb temperature reaches the gelation point, which may be two hours or more after drying has been started. After two and one-half hours of dehydration of unblanched potato, starch gelation may be seen in cells about 500 μ from the surface, while the surface cells still retain intact starch grains (Fig. 10). The evaporation retards the rise of temperature in the surface regions. Further dehydration results in progressive gelation toward the surface, but even after three and one-half hours of drying at 90°C.(194°F.) there may still be intact starch grains present. Raw potato dried at temperatures near or below the gelation point will contain starch which is mostly composed of intact, ungelled grains.

In blanched potato the shrinking of the cells is limited by the gelled starch. The diameters of normal cells in fresh potato average 250 to over 500 μ (Fig. 6). Completely dehydrated cells of blanched potato are about 30 by 100 μ (Fig. 7). Blanched, dehydrated potato develops a greater hardness than does dehydrated carrot because of the gelled starch content. The potato texture becomes brittle and glassy. As in carrots, an outer case is formed during early stages of drying; but a completely dehydrated, blanched potato cube is much harder than the case formed in the early stages of drying. Case hardening, then, is only relative to the thickness of the cube or slice and is conditioned by the rate of early evaporation.

A white chalkiness, generally characteristic of unblanched, dehydrated potato, is caused by intact starch grains on the surface. This, of course, may extend to some depth, depending upon the temperature of drying and internal starch gelation. Case hardening in unblanched potatoes is mostly an effect produced by the crowding and compacting of the outer and intact starch grains.

It was found that blanched, dehydrated potato sometimes develops a chalkiness during or after dehydration. Several trials were made of blanching conditions and the results indicated that with shorter periods of blanching, or incomplete exposure to steam, not all the starch contents of the tissues may be gelled. These starch grains may remain intact if, during dehydration, the temperature of the potato does not reach the gelation point. Depending upon the thickness of the slices of potato, three to six minutes of steam blanching will gel the starch throughout the tissues.

In the development of the outer case of blanched potato during dehydration (Fig. 11) the inner tissues show gelled starch in which trapped gases occur and appear black through the microscope. In the severely shrunken cells of the case the gases have been forced out of the gelled starch.

The effects of reconstitution upon blanched and unblanched potato samples after dehydration may be quite similar although caused by entirely different characteristics of the dried products. Unblanched, dehydrated samples with ruptured walls and loose surface starch frequently are pasty and lumpy when reconstituted. The presence of both forms of starch may also produce lumpiness in the cooked product. Forced drying may "cook out" the centers of potato pieces as it does with carrots, and

this also produces an undesirable product when reconstituted. All of these effects tend to be more pronounced in thicker slices and cubes.

SUMMARY

Preparation of the vegetable tissues for microscopic examination after different periods and techniques of drying was accomplished by a modified celloidin technique by which the dehydrated condition was preserved. The extent of shrinkage during dehydration, and the differences between blanched and unblanched, dehydrated vegetables have been described, using carrots and potatoes as types of low-starch and high-starch vegetables, respectively.

Case hardening is conditioned by rapidity of evaporation, cell contents, and the thickness of the slices or cubes prepared for drying. Blanching of potato causes starch gelation and the gelled starch seals the dehydrated tissues.

Incomplete gelation of starch contents causes lumpiness in the reconstituted product. Forced drying also produces other undesirable effects.

Because of the more uniform rate of evaporation obtained and because of uniform starch gelation, steam blanching of thin pieces of vegetable is desirable prior to commercial dehydration. Other reasons for blanching are enzyme inactivation and chemical changes which are not discussed in this paper.

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MICROSCOPY OF THE OILS AND CAROTENE BODIES IN DEHYDRATED CARROTS[1]

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The development of "off" odors in dehydrated carrots constitutes one of the problems encountered in the storage of the dried product. Changes in odors arise as a result of chemical change during the blanching and drying processes and over periods of storage. Carotene, essential oils, and lecithins are directly involved in the alterations produced by any of the present commercial methods of dehydration.

The vegetable oils (including lecithins) in the cytoplasm of plant cells deserve serious consideration in research devoted to improving the quality of dehydrated foods. In general, these oils may occur free or weakly combined with proteins of the cytoplasm. The association of lipoids with proteins is generally referred to as a lipoprotein complex. This association is readily destroyed by denaturation of the cytoplasm, and the lipoidal substance is then freed. Under certain conditions the freed oils in dried vegetables oxidize and fatty acids may be liberated. Such oxidation is related to methods of storing and to the preservation of carotene and other oil-soluble vitamin substances.

Denaturation of the cytoplasm, which contains the carotene and oils, results from heating, drying, or freezing treatments. Vegetables dried by heat are usually blanched by steam or hot water scald before dehydration but are sometimes dried raw; in either case, they undergo changes described as denaturation. While the general effects of denaturation are similar for the various methods used, the relationship of the oils and carotene is variously affected.

The purpose of this study on the histological aspects of carrot dehydration was to establish microscopic evidence of the relationships between carrot oils and carotene in both fresh and dehydrated tissues. The observations are interpreted with particular reference to those factors concerned with "off" odors and carotene loss.

TECHNIQUE

Histological technique consisted of sectioning fresh carrot tissue on the freezing and sliding microtomes. Dehydrated samples were reconstituted by cooking and then were sectioned on the freezing mocrotome; some were cut without reconstitution on the sliding microtome. Both fresh and dehydrated carrots were also treated with Flemming's solution to preserve the lipoids and then were prepared by the paraffin method for sectioning on the rotary microtome.

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Paraffin sections were stained and mounted in Canada balsam; others were mounted in water, glycerin, or glycerin jelly after staining. Observations were also made on samples of commercially prepared carotene. The effects of heat and drying on both oils and carotene bodies were studied with the polarizing and the ordinary biological research microscope. Sudan IV and other fat stains were used to render the oil bodies more photogenic. Delafield's stain was used for cell walls and cytoplasm. Other techniques and methods of preparation are discussed later.

DISTRIBUTION OF LIPOIDS AND CAROTENE

A brief review of the anatomy of the carrot is necessary to a general understanding of the location of the oils and carotene bodies. The inner core of the carrot consists of scattered groups of xylem elements surrounded by thin-walled parenchyma. At the periphery of the core the xylem occurs in radial rows that are more pronounced in the upper regions of the storage organ. The outer cylinder of the carrot, separated from the core by the cambium, consists mostly of phloem parenchyma and pericyclic parenchyma. A thin layer of cork and cork cambium, collectively the periderm, forms the outermost layers. Esau (1940) has discussed the anatomy and histological development of the carrot in detail.

Horizontal and vertical oil ducts traverse the phloem region. The cells surrounding these ducts are smaller than the rest of the parenchymatous cells. Most of the carotene appearing in granular form is found in the cells of the phloem region. Some granules are found in the cambium and the parenchyma of the core. Carotene is also diffused throughout the cytoplasm, and Weier (1942) has found it associated with the cytoplasm surrounding the starch grains.

The "fixed" oils of the fresh carrot are finely dispersed and occur mostly in a lipoprotein complex. Sections cut from tissues treated with osmic acid show many minute lipoidal bodies in the cytoplasm. Essential oil is also present, but in fresh sections only a very few scattered droplets may be found. Some of the carotene bodies take on additional color when fresh sections are stained with Sudan IV and suggest a lipoidal association. Weier (1942) found that carotene is associated with some other substance in plant cells and demonstrated its solution in lipoids when the tissues were heated or dried.

Richter (1909) reported that ethereal (essential) oil from the fruit of carrots contained free palmitic and isobutyric acids. This leads one to wonder if his extraction methods did not free oils from the lipoproteins, with the result that more than one kind of oil was analyzed. He also reported the presence of pinene which belongs to the terpene group, characteristic constituents of essential oils that usually do not contain these fatty acids. Some of the aged, unblanched, dehydrated carrot samples develop an "off" odor suggestive of a fatty acid rancidity. Also, the ionone (violet) odor arising from natural carotene breakdown, and sometimes noticed in freshly cut carrots, is present in dehydrated samples and suggests that some of the "off" odor is related to carotene degradation. This violet odor may become more pronounced upon storage and aging of the dried product.

The relationship of plastid pigments to lipoproteins has been investigated by Guilliermond, Mangenot, and Plantefol (1933), by Guilliermond

(1941), and by Strain (1938). Guilliermond (1941) believed that carotene and starch are first formed in lipoprotein bodies (mitochondria) and that later the starch and lipoprotein withdraw and leave the carotene in a free, granular state. Mitochondria are generally considered to be highly hydrated lipoprotein bodies of greater viscosity than the surrounding cytoplasm, but their relationship to plastid formation remains controversial. These subjects are reviewed in detail by Newcomer (1940). The facts pertinent to this study are that there are lipoproteins present in the cytoplasm and that carotene, as well as other plastid pigments, shows some relationship to them.

EFFECTS OF COOKING AND DEHYDRATION TREATMENTS

The effects of drying upon carotene in fresh tissues, described by Weier (1942), indicate a lipoidal association. Similar effects were observed in the commercially dried carrot samples. Some of the carotene granules apparently are quite lipoidal and become globular when the tissues are heat dried and then reconstituted. Commercially prepared carotene crystals did not become globular when gently heated, but when higher temperature—100°C.(212°F.) or more—was used, the oxidation of the carotene proceeded more rapidly and some of the crystals assumed semiglobular forms. Changes in the lipoprotein complex in carrot tissue may be induced by cooking, drying, or freezing. When sliced fresh carrot is cooked, the lipoprotein association is destroyed and the freed oils coalesce into tiny globules in the cytoplasm. These droplets are scattered throughout the tissue and frequently appear in minute, bead-like strands (Fig. 1). They are more profuse in the oil-duct regions and range in size from barely visible specks to a few microns in diameter. Prolonged cooking produces larger globules by coalescence of the smaller ones.

Alcohol, ether, and other fat solvents dissolve the oil globules readily when drawn under the cover glass over the sections. The color varies from nearly colorless for oil alone to light orange for oil in which carotene has been dissolved. The minute, bead-like strands of oil droplets strongly suggest that the oil is derived from a lipoprotein complex in the cytoplasm.

The breakdown of the lipoprotein complex does not appear to be related to a specific temperature other than that at which denaturation takes place. Sections of fresh carrot cut on the sliding microtome and allowed to dry at room temperature contained numerous minute oil droplets after they were soaked in water. Other fresh sections were treated as follows: (1) kept moist at 35, 55, and 75°C.(95, 131, and 167°F.) for periods up to one-half hour; and (2) allowed to dry on the slides at the same temperatures and time intervals as the moist ones. After a half hour of treatment the moist sections of the 35°C. lot did not show any appreciable differences from fresh, untreated sections. The moist sections of the 55 and 75°C. lots, however, contained numerous oil droplets similar to those produced by cooking. The droplets became more abundant in proportion to both time and temperature increases. All of the dry sections, after soaking, contained many tiny droplets.

When reconstituted by cooking, commercially dehydrated carrot samples contain many oil droplets ranging in size from less than one to over

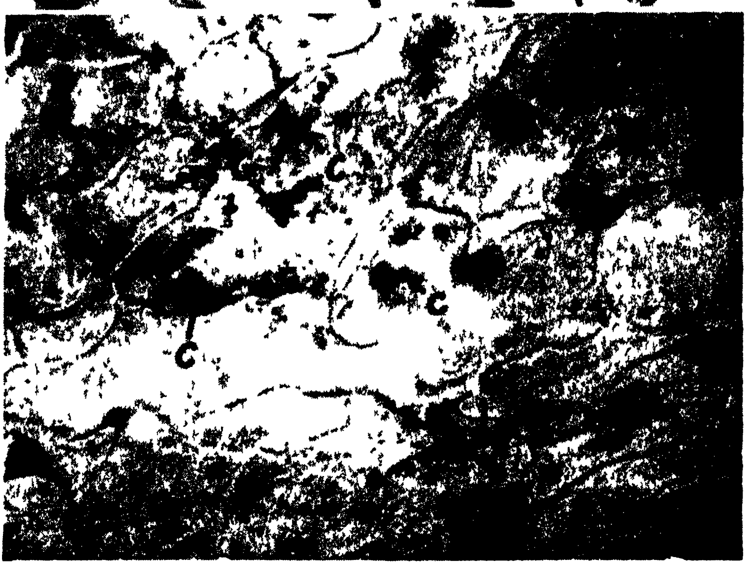


FIG. 1. Photomicrograph of section cut from cooked carrot tissue. G—globules of oil freed from lipoproteins. Sections stained with Delafield's stain and Sudan IV. (Magnification 500 ×.)

FIG. 2. Photomicrograph of unstained section cut from blanched, dehydrated carrot and showing: G—oil globules; C—carotene granules. (Magnification 400 ×.)

10 μ in diameter, and there appear to be no differences in numbers of these droplets between samples that were blanched prior to dehydration and those that were not (Figs. 2, 3, 4). The globules are distributed throughout the tissues but are more abundant in the oil-duct regions. Unstained sections show ranges in coloration similar to those found in freshly cooked carrot. There tends to be more coloration in the globules of the dried samples, however, and the blanched samples appear to have more of the carotene-colored droplets than do the unblanched ones.

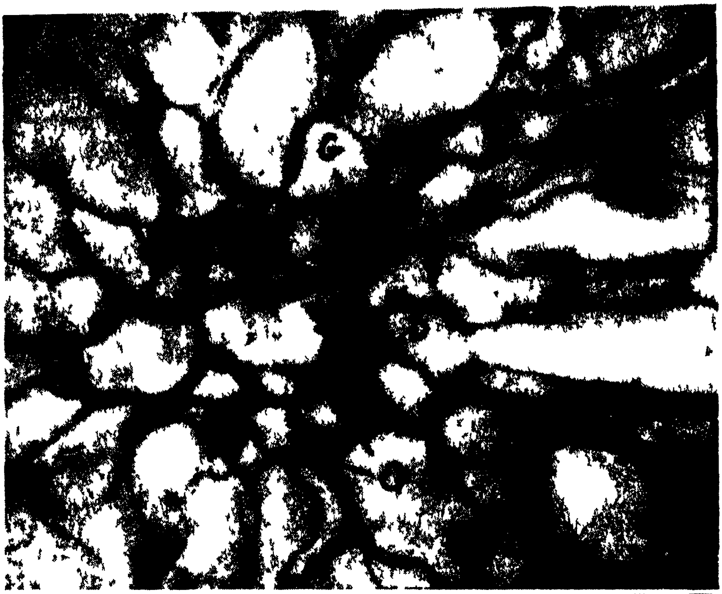
When sections of the reconstituted carrot were stained with Sudan IV and allowed to dry on the slides it was possible to observe the physical alteration of the freed oils during the drying process. The globules coalesced and formed a thin film over the intracellular surfaces. After drying, some sections were soaked in hot, and others in cold, water. After cold-water soaking large, irregular oil globules were formed; and after soaking in hot water smaller, spherical globules were formed which were even more intensely colored with dissolved carotene than those observed before drying.

Sections cut from dehydrated samples without reconstitution showed the same sort of oil film. In blanched samples this film was more deeply colored with dissolved carotene than in unblanched samples. This deeper color of oil in blanched samples indicates that after lipoprotein breakdown by blanching, there is more time for the carotene to go into solution during dehydration than there is in unblanched samples in which lipoprotein breakdown takes place after drying begins. With low-temperature methods of drying, denaturation of the cytoplasm occurs either upon freezing before dehydration or when the tissues have lost sufficient water. Thus, in any drying treatment, unblanched carrots will contain less oil-dissolved carotene than will blanched ones, and the quantity will be in proportion to the mobility of the substances during the drying process.

Not all of the carotene goes into solution, and therefore carotene granules are present in both cooked and dehydrated samples (Figs. 2 and 4). The fat solvents applied to dissolve the oil droplets also readily dissolve the carotene in the dried, reconstituted samples. Fresh, unstained sections, however, may retain some of their carotene granules for several minutes after the solvents have been applied. The semiglobular carotene bodies in dried, reconstituted sections dissolve as rapidly as do the oil droplets. Dilutions of alcohol up to 75 per cent appear to have less effect upon the carotene in both fresh and reconstituted samples than do more concentrated alcohol solutions. After one-half hour in 50 per cent alochol, sections of carrot tissue still contained some granular carotene.

Differences in carotene content between blanched and unblanched samples are visible after short periods of storage in air. The unblanched samples fade more rapidly than do the blanched ones. Analyses made of blanched and unblanched samples stored in air-containing jars for six months revealed only 9.36 mg. of carotene per 100 grams of the dry, unblanched samples as contrasted with 53.8 mg. of carotene per 100 grams of the dry, blanched samples.[1] Associated with this striking difference were the more pronounced "off" odors in the unblanched samples.

[1] These carotene determinations were made by Dr. H. J. Dutton and Mr. G. F. Bailey of the Western Regional Research Laboratory.



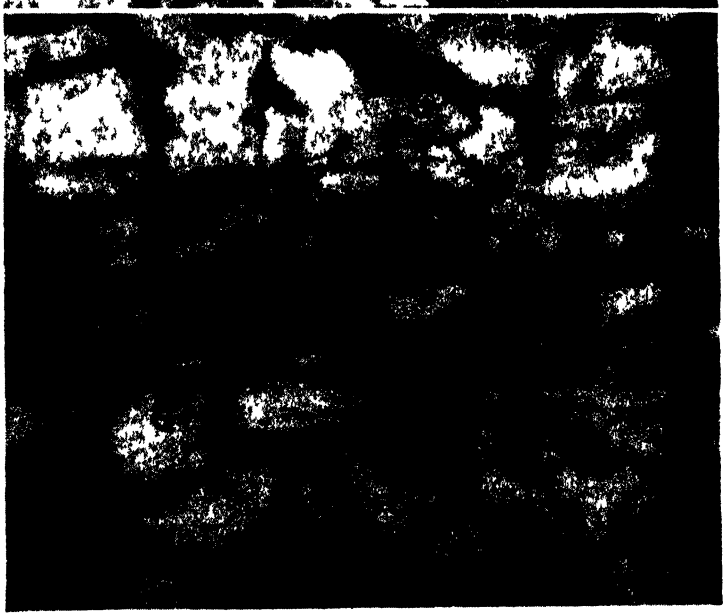


FIG. 3. Photomicrograph of section cut transversely through an oil duct in the phloem region of blanched, dehydrated carrot after reconstitution. G—oil globules; D—oil duct. Stained with Delafield's stain and Sudan IV. (Magnification 400 X)

FIG. 4. Photomicrograph of section cut longitudinally through the cells associated with an oil duct in blanched, dehydrated carrot after reconstitution. G—oil globules, C—carotene granules. Stained with Delafield's stain and Sudan IV. (Magnification 400 X.)

Carrot tissue that had been frozen and vacuum-dried was decidedly lower in carotene content after two months of storage in air than were blanched, heat-dried samples stored in air for the same period of time. The vacuum-dried samples also had more pronounced "off" odors than even the unblanched, dried carrot of the same age and storage. When soaked in cold water and sectioned, the vacuum-dried sample contained only a few visible oil globules, but when reconstituted by cooking, sections of this sample contained numerous oil droplets. No filming with oil could be detected in dry sections of the frozen, vacuum-dried carrot. It seems likely, then, that this method does not permit sufficient mobility of the cytoplasmic substances to allow oil filming and carotene solution in the oils to occur to any appreciable extent.

SOME ASPECTS OF CAROTENE PRESERVATION

It is a well-established fact that carotene preservation can be effected by dissolving the carotene in fixed oils, and the evidence from these microscopic studies is in accord with that fact. An interesting correlation of these observations with analytical results for carotene content immediately after dehydration is shown in the comparison of blanched with unblanched samples. Blanched carrot contained 183 mg. of carotene, while unblanched carrot of the same drying run contained 152 mg. of carotene per 100 grams of the dry samples. Thus, since the carotene is not as effectively protected by oil during dehydration of unblanched carrot, some initial oxidation of it does occur. There appeared to be no pronounced correlation between carotene contents of dried carrot samples of different final moisture contents, whether samples were blanched or unblanched; this was probably because the carotene is oil soluble and not water soluble.

The problems arising from "off" odors apparently cannot be solved by blanching alone. Since carrot oils contain at least one unsaturated fatty acid, Richter (1909), it is reasonable to regard the freed oils in the root (after dehydration) as a source of future "off" odors. At the same time it is also evident that even in blanched, dried carrot, carotene loss occurs over a period of time.

One of the methods employed to minimize oxidation in commercially dried vegetables is storage in an inert gas, such as nitrogen. The results of this practice and of other treatments such as sulfuring have not been studied histologically. There are possibilities that less expensive procedures might be utilized to effect carotene retention and to minimize "off" odors. These are suggested by the behavior of the starch content in young carrots when they are dried after blanching. Young carrots contain abundant starch in the phloem regions. As the carrot matures in the field most of this starch is utilized in metabolic processes of growth. At harvest time, fully grown carrots contain only small amounts of granular starch.

When young carrots are blanched, and then dehydrated in a tunnel drier such as described by Chace, Noel, and Pease (1941), the starch which has been gelled by blanching seals the tissues together. Completely dried samples are hard and brittle and show less carotene loss and the "off" odors are less pronounced than in older, dehydrated carrot.

In connection with the study of starch and carotene in young carrots, comparisons have been made with dehydrated sweet potatoes. The latter contain carotene granules as well as abundant starch. Lecithins are also present. When blanched and dehydrated, sweet-potato tissues contain freed oils and carotene granules effectively sealed in by the dried, gelled starch. In blanched, dried sweet-potato samples stored two months in air-containing jars there were no noticeable "off" odors, and carotene loss was minimized. The starch is distributed throughout the sweet-potato tissue, however, while in young carrots it is localized and many of the cells do not contain a sufficient amount to provide an effective sealing when blanched and dehydrated. Studies have been made on other vegetables, including parsnips, with reference to the role of starch in the retention of carotene and prevention of "off" odors. Briefly, the parsnip is rich in starch at the end of the first year of growth. It does not develop "off" odors when blanched and dehydrated and stored in air-containing jars for several weeks. Some blanched and dried samples have been stored four months in air without marked loss of the fresh, natural odor, but unblanched, dehydrated samples stored a like period in air developed "off" odors in a few weeks.

The function of starch as a protective mechanism in dehydrated sweet potatoes and parsnips leads to consideration of dextrins and pectins as possible substitutes for starch in nonstarchy vegetables. The protective properties of starches and dextrins, as well as other gels, have been known to colloid chemists for a number of years. Blitz and Truthe (1913), Gutbier and Weingärtner (1913-14), Visez (1922), and others have investigated the various protective colloidal properties of dextrins and starches.

Dextrin and pectin dipping prior to dehydration was tried experimentally to compare its effects with those of blanching as exhibited by the dried product. Sections cut from dried carrot that had been blanched and soaked one-half hour in three per cent dextrin showed the dextrin diffused throughout the tissue; it was not possible to demonstrate its adsorption on intracellular surfaces because of the visual limits of the microscope. There is, nonetheless, reason to assume that such a mechanism does occur. Raw carrot slices soaked 10 minutes in a .5-per cent dextrin solution and dried without blanching retained 18 per cent of their original carotene content after four weeks of storage in oxygen. Raw slices soaked similarly in a .1-per cent pectin solution and dried without blanching retained 21 per cent of their original carotene content after the same storage. On the other hand, unblanched and undipped slices that were dried and stored four weeks in oxygen had retained only 11 per cent of their carotene.

These results are not striking when compared with carotene retention of about 60 per cent in blanched, undipped carrot stored for the same length of time in oxygen after dehydration. The fact that pectin dipping of raw slices had virtually doubled carotene retention by unblanched, dehydrated carrot, however, suggests its use on blanched vegetables. In this connection it is well to emphasize that raw vegetables contain living cytoplasm which has selective permeability and will not "soak up" the solutions in sufficient amounts to provide an effective intracellular adsorption. The denatured cytoplasm in blanched carrot slices offers relatively

little resistance and the solutions can diffuse into the tissues until an equilibrium is established.

At present there is insufficient information on pectin dipping of blanched carrot to make any comparisons with the practice of nitrogen storage. Additional consideration must be given to the fact that some pectins are not sufficiently stable to retain gelling properties during dehydration, and thus would not effectively seal the tissues. It seems reasonable to conclude, however, that the protective mechanism would be supplied by adsorption on the intracellular surfaces throughout the tissue in blanched carrot but only superficially in unblanched carrot. With a stable pectin the gel state would not be altered during dehydration and the drying of the pectin gel would be comparable in sealing action to that of gelled starch in blanched, starchy vegetables.

SUMMARY

The fixed oil of carrot occurs in the fresh tissues in a lipoprotein association. Blanching and dehydration break down the lipoprotein and free the oil in which some of the carotene dissolves.

Some of the "off" odors of dehydrated carrot are related to oil and carotene oxidation. Preservation of carotene in the blanched, dehydrated carrot is partially effective according to the amount of carotene dissolved in and coated by the oil, and also according to the rate at which the oils oxidize. Unblanched, dehydrated carrots contain less oil-protected carotene. Blanching causes earlier and more complete liberation of oil, which coats and protects the carotene, because of the greater length of time in which it can occur and also because of the greater mobility of substances in the denatured cytoplasm.

Dipping in weak solutions of dextrin and pectin prior to dehydration increases the carotene retention in unblanched carrot, but such protection afforded by the pectin is only superficial.

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CHANGES IN TISSUE COMPOSITION IN DEHYDRATION OF CERTAIN FLESHY ROOT VEGETABLES[1]

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The phenomenon of "case hardening" of starchy and nonstarchy vegetables was described and the relationships of carrot oil and carotene in dehydrated carrots were discussed in previous papers by Reeve (1943a,b). It is well to re-emphasize that the significance of vegetable oils to problems of commercial vegetable dehydration deserves serious consideration. The oils may be involved in the oxidation mechanisms and the development of "off" odors in certain of the dehydrated products, particularly during storage. Not only are there special cytoplasmic bodies of lecithin or vegetable-oil nature, but there are also lipoproteins within the rest of the cytoplasm, Newcomer (1940). Although it must be admitted that these oils constitute only a small percentage of the fresh weight of the vegetable tissues, their concentration and oxidation occur as a result of drying.

The vegetables with which present observations are concerned included beets, parsnips, rutabagas, turnips, and sweet potatoes. Anatomical characteristics of these are discussed by Artschwager (1926), Hayward (1938), and Winton and Winton (1935).

HISTOLOGICAL METHODS

Preparation of the dehydrated vegetables for microscopic studies consisted of reconstitution by soaking in water or by cooking, and then sectioning the reconstituted product on the freezing microtome. Some sections were cut on the sliding microtome, either in the partially reconstituted or in the dehydrated condition after the samples had been embedded in celloidin as previously described, Reeve (1943a). Sections of fresh tissues were prepared both in the fresh condition and by the paraffin method of histological technique. These were utilized for comparative studies. Other techniques are discussed with reference to particular purposes for which they were employed.

CHANGES RESULTING FROM DEHYDRATION OF BEET SLICES

The anatomy of the sugar beet is discussed in detail by Artschwager (1926). Any histological distinctions between the sugar beet and the red beet, significant to dehydration, are confined to cell contents. The tissues of the vascular rings that make up the structure of the beet root consist of lignified xylem cells, cambium, phloem, and parenchyma. The lignified xylem tends to retain its structure during dehydration, while the other tissues undergo extreme shrinkage. The resulting configuration in dehydrated slices is a series of slight ridges alternating with depression of collapsed cells. The development of the xylem is not pronounced in young

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beets; consequently dehydration of these does not result in such sharply defined ridges as with older beets. Further development of the xylem and its lignification produces woody quality in the older beet and renders the vegetable undesirable for food. According to Winton and Winton (1935), the hydropectin content of beets ranges from 25 to 50 per cent of the dry solids. The Ruthenium Red test for pectins, described by Branfoot (1929), was used on sections of fresh tissue prepared by the paraffin method. The pronounced staining indicated a high pectin content of the cell walls and middle lamellae. The same test on sections of reconstituted beets prepared the same way showed very little loss of these pectins as indicated by reduced staining, except in sections cut from samples that were overcooked.

The glucoside "coniferin," which occurs in the cambium of conifers, is also found in beet roots. It forms fine crystals, often stellate in pattern. Sections cut from reconstituted samples of dried beet contained many of these finely divided stellate crystals (Fig. 1). Similar crystals may be observed in the fresh tissues. According to Winton and Winton (1935), lecithin, a number of fatty acids, and choline are also present in beet roots. Whether or not the glucoside crystals are the source of the sterols frequently reported for beets was not determined, but microchemical reactions with antimony trichloride were found to be similar to those obtained with sterols and saponins. Some authorities classify the glucosides with plant sterolins.

Blanched, dehydrated beets generally retain their color and natural odor for several weeks. It is possible that these keeping qualities are due to the high pectin content. The action of natural pectins as a sealing mechanism in the dehydrated tissues was previously suggested, and the effect of pectin dipping on unblanched carrot was discussed, in another paper, Reeve (1943b). Since the oil of beets contains several fatty acids, it would be expected that oily odors might develop after several weeks' storage, similar to the oily odors developing on storage of dried carrot. That such an odor does not occur is reasonable evidence of some sort of protective mechanism.

When sections of reconstituted beets were stained with Sudan IV, only occasional oil globules could be detected. Sections of freshly cooked beets also contained few oil droplets, although it is safe to assume that cooking, blanching, or drying had freed oils from the lipoproteins in the cytoplasm. Further studies revealed the particularly interesting fact that in the freshly cooked and reconstituted samples alike, the oils were adsorbed on the surfaces of the glucoside crystals. These crystals appeared to stain with Sudan IV in these samples, but not in sections of fresh beet tissues. Since coniferin is not soluble in ether, it was possible to demonstrate the adsorption of the oils on the crystal surfaces. Sections of cooked and of reconstituted samples soaked in ether before staining showed no oils present. Sections first stained in Sudan IV and then washed with ether revealed that the oils on the surface of the crystals were removed by the ether. Whether or not this oil adsorption and its relationship to the glucoside provide an additional antioxidant mechanism could not be determined, but such a possibility might account in part for the better keeping quality of the dried, stored product as compared with carrot.

Other crystal inclusions are found in the phloem regions (Fig. 2). Esau (1934) has described the phloem of the sugar beet and has cited literature in which crystals in the phloem have been regarded as carbohydrate in nature. Such crystals occurring in the fresh tissues would almost certainly dissolve as a result of either the blanching or the reconstitution processes. The crystals shown (Fig. 2) do not react to any carbohydrate tests applicable in histological techniques, and further identification of them proved uncertain. They stain light brown with IKI, but do not respond positively to other glycogen tests. Large crystalloid bodies are found in the cells of many other plant species and have not been chemically identified.

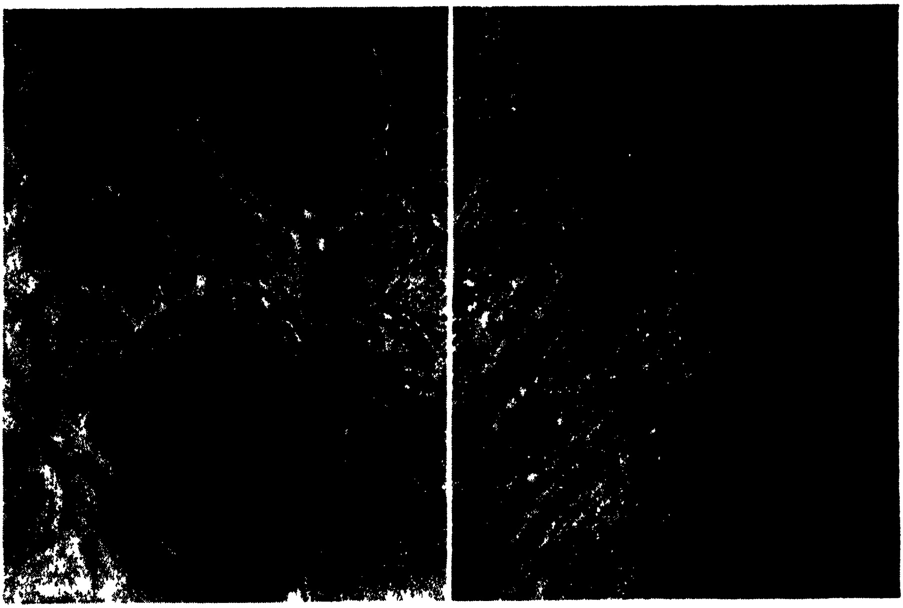

FIG. 1. Parenchyma cells of reconstituted (blanched and dried) beet showing: C—glucoside crystals; W—cell wall; P—plasmolyzed cytoplasm. Stained with Sudan IV (200 ×).

FIG. 2. Section showing: G—granules in the phloem region of beet, and W—wall (200 ×).

The preservation of color constitutes a major problem in the dehydration of beets. Betanin, the anthocyanin pigment responsible for the color of red beets, occurs in solution in the cell sap. It also may provide some antioxidant effect in the stored, dehydrated product. Denaturation of the cytoplasm of the cells by cooking or by drying destroys its selective permeability and the pigment readily diffuses out of the tissues when the treated slices are placed in water. The same happens when beets are frozen. Sliced beets blanched by water lose considerable color. Steam blanching of sliced beets results in less pigment loss, but there is considerable loss of the water-soluble pectin by either treatment. When steam blanched, slices of beets tend to stick together during dehydration and thus may not dry uniformly.

This is because the water-soluble pectins ooze out and tend to seal the slices together during dehydration.

Steam or hot-water scalding of whole, unpeeled beets solves most of the problems of pigment loss and contributes to uniform drying of the product. The pectins are not as severely affected and there is less sticking together of the slices while drying. During reconstitution of dried beets, however, there is an inevitable loss of pigment. The best results are obtained when whole beets are blanched, then peeled and sliced thinly (one-fourth inch or less), and dried so that the product will reconstitute rapidly in a minimum amount of water before pigment loss becomes pronounced. Because increased woodiness of older beets necessitates longer cooking time to make tender, only young, nonwoody beets should be used for commercial dehydration. These, when properly prepared, will reconstitute rapidly and with a minimum of color loss.

CHANGES IN CELL CONTENT RESULTING FROM DEHYDRATION OF PARSNIPS

In contrast with carrots in which the starch present in young roots is utilized in later summer growth, the parsnip retains its starch over the fall and winter months. For growth during the next spring and production of the seed stalk in summer, the starch of the parsnip is utilized as a reserve food source. Most of this starch is found in the outer cylinder of phloem and pericycle, but it is more generally distributed in parsnip than in carrot. The inner core of the parsnip, as in carrot, possesses strands of lignified xylem cells interspersed with parenchyma cells. In parsnip some starch may be found in the parenchyma of the core. Both vegetables belong to the Umbelliferae and contain characteristic essential oils. Carotene granules are few in parsnip, but the carotene present shows relationship with the oils and lipoproteins similar to that found in carrot. Blanching and dehydration result in freeing oil from the lipoproteins, and the carotene then becomes dissolved in or coated by the oil. In sections of dehydrated, reconstituted carrot the oil and carotene bodies may be seen (Fig. 3). In parsnip, however, the starch is gelled by blanching, and sections cut from reconstituted parsnip show the carotene and oil droplets masked by and embedded in the gelled starch (Fig. 4). The gelled starch renders the cell content somewhat granular and grayish in appearance when photographed.

The problem of "off" odors do not appear to be as critical in storage of dehydrated parsnip as they are for dehydrated carrot. Blanched, dehydrated parsnip retained a fresh-parsnip odor after three to four months of storage in air-containing jars. Unblanched parsnip dried at temperatures sufficient to gel the starch also maintained a natural odor, but with samples dried at temperature below the gelation point of starch there was a noticeable oily odor after a few weeks of storage in air. As determined experimentally, parsnip starches gel at 70 to 75°C.(158 to 167°F.).

It seems that the lack of "off" odors in stored, dried parsnips in which starch gelation has occurred may best be attributed to the protective role of the gelled starch which seals the tissues together during dehydration and retards the oxidation processes. Since it is assumed that blanching

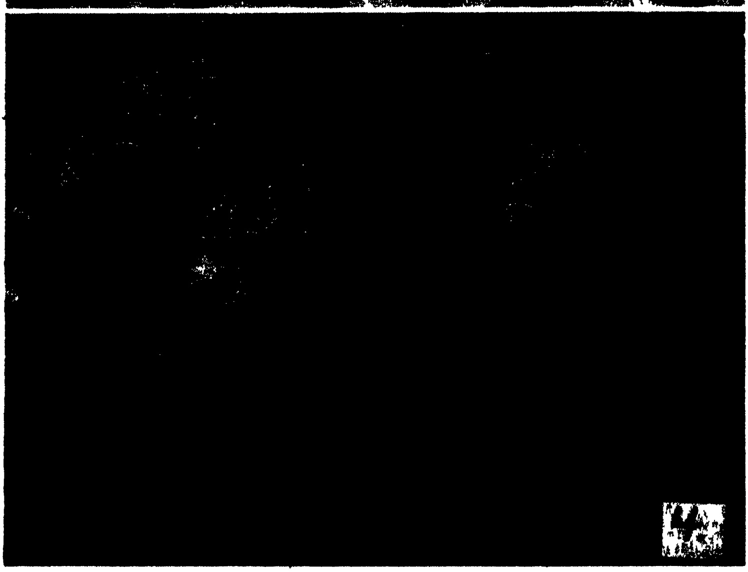

Fig. 3. Carrot tissue from reconstituted, blanched and dried sample, D—oil duct; G—oil globules. Note absence of starch (200 ×).

Fig. 4. Section of reconstituted (blanched and dried) parsnip, D—oil duct; G—oil globules; S—gelled starch; W—cell wall (200 ×).

inactivates the enzymes present, any oxidations that do occur are probably auto-oxidations which can be inhibited by mechanical exclusion of oxygen. Most likely both enzymatic and auto-oxidations occur in unblanched vegetables, but both would be slowed down by the sealing action of gelled starch if gelation does occur.

FRESH AND DEHYDRATED TISSUES OF RUTABAGA

There are no differences in anatomy between rutabaga and turnip that are significant in dehydration. Cell contents, however, may vary according to the different varieties of each. Rutabagas range from white to yellow in color and show corresponding cytological differences in pigment content.

FIG. 5. Section from reconstituted (blanched and dried) rutabaga, G—oil globules; P—plasmolyzed cytoplasm; W—cell walls (200 X).

Turnips show similar variation in color, and in addition, possess a red-purple pigment in the outer tissue layers of the upper portion of the root. These layers are removed by peeling. The yellow forms of turnip, as well as of rutabaga, possess slight amounts of carotenoid granules and are commonly referred to as rutabagas. The young vegetables may be relatively high in starch, but the older roots contain little starch. Both vegetables are high in sucrose and dextrose, and contain lecithins and other cytoplasmic compounds.

The oil globules formed as a result of lipoprotein breakdown during blanching or drying of rutabaga or turnip are not as abundant as in carrot tissue (Fig. 5). There is relatively little carotene, however, and thus what is present is rather effectively protected by solution in or coating by the freed oils. Since these vegetables are essentially nonstarchy, the problems of storage are similar to those for carrots. After several weeks of storage in air-containing jars, dehydrated rutabagas developed slightly oily odors

in addition to the pronounced natural odor. It does not seem likely, therefore, that the sugar content provides any antioxidant mechanism during storage, and usually, the vegetables large enough to be used as food and still young enough not to be woody, contain little starch. Under these circumstances, it might be practical to use a pectin dip as a substitute for naturally occurring starch, as previously suggested by Reeve (1943b), to reduce the oxidation of the oils during storage of the dehydrated products.

ROLE OF STARCH IN DEHYDRATION OF SWEET POTATOES

It was previously pointed out that stored, dehydrated sweet potatoes, blanched before drying, do not develop "off" odors such as occur upon storage of dehydrated carrot, Reeve (1943b). This fact is definitely correlated with the starch content of sweet potatoes and the gelation of the starch by blanching. The behavior of starch in parsnip blanching and dehydration further indicate such a correlation. With certain dehydration treatments, however, starch gelation does not occur.

When fresh sweet potato is frozen and dehydrated by vacuum, the starch grains may be ruptured and their content of granular starch dried without gelation. Consequently, the intracellular surfaces are increased and there is no protective sealing of these surfaces. In addition, ice-crystal rupturing of the tissues takes place if the freezing process is slow. The dehydrated tissues are then filled with numerous pockets that further expose the contents to oxidation.

Vacuum drying does not result in extreme shrinkage of the tissues; the vacuum-dried vegetables tend to remain quite spongy. Such samples stored in air-containing jars for four or five weeks develop oily, rancid odors and fade considerably. As was stated for carrots, Reeve (1943b), frozen, vacuum-dried samples lack effective oil protection of the carotene. The oils freed from the lipoproteins remain too finely dispersed in the vacuum-dried products to coat or to dissolve much of the carotene. As a result, carotene loss and oil oxidation occur readily upon storage in air.

Dehydration of blanched sweet potato produces an entirely different relationship between the starch, oil, and carotene as compared with vacuum drying. The resulting product is hard and glassy and does not develop "off" odors even after several months of storage in air-containing jars. During dehydration of sweet potatoes by heat, the starch gelled within the cells by blanching is compressed by the shrinking of the cell walls and seals the tissue in the same manner as was described for white potatoes, Reeve (1943a). The oils freed by lipoprotein breakdown and the carotene granules are embedded within these starch masses. Air, or other gases that might be present, is forced out by the compression of the tissues. The completely dehydrated product is relatively impervious to air. When reconstituted, sections of these samples show the oils and carotene still embedded in the swollen, gelled starch (Fig. 6) in the same manner as in sections of reconstituted parsnip (Fig. 4).

Analyses of blanched, dehydrated sweet potato and blanched, dehydrated carrot show significant differences in carotene retention after storage. Immediately after drying, sweet-potato samples contained 19.5 mg. of carotene per 100 grams of the dry sample and carrot samples contained

140 mg. of carotene per 100 grams of the dry sample.[1] After eight weeks of storage in oxygen, the sweet potato contained 11.6 mg. and the carrot 36.5 mg. of carotene per 100 grams of the stored dry samples. Thus, the starchy vegetable had lost about 40 per cent, and the low-starch vegetable 60 per cent, of the original carotene content. After eight weeks of storage in air, the sweet potato contained 17 mg. and the carrot contained 82 mg. of carotene per 100 grams of the dried, stored samples. In other words, upon air-storage the starchy vegetable retained 90 per cent and the low-starch vegetable retained only about 60 per cent of its original carotene content.

The protective properties of the gelled starch are further emphasized by the nature of unblanched, dehydrated sweet potatoes. If the temper-

FIG. 6. Reconstituted (blanched and dried) sweet potato, C—carotene granules; G—oil globules; S—gelled starch; W—cell wall (200 X).

ature of dehydration is below the gelling point of starch, the dried product will contain granular starch. The surface areas within the cells have not been decreased, and the shrinking of the cells has been limited by the masses of solid starch grains. Thus air remains within the tissues. Such samples fade and become oily after only a few weeks of storage in air, and their carotene loss is comparable to that of unblanched, dried carrot, Reeve (1943b). If the dehydration temperature is only slightly above the gelling point of starch, the wet-bulb temperature may not reach the gelation point before the samples are dried. Thus there may be little or no starch gelation and such samples undergo the changes in odors and carotene content just described.

[1] These carotene determinations were made by Dr. H. J. Dutton and Mr. G. F. Bailey of the Western Regional Research Laboratory.

As discussed with reference to carrot oils, Reeve (1943b), lipoprotein breakdown does not occur in unblanched vegetables until the tissues have dried sufficiently to denature the cytoplasm. The result is that in both carrot and sweet potato, there is less time for the carotene to dissolve in or be coated by the freed oils than when the vegetables are blanched before drying. While it might seem desirable to remedy this by drying at a sufficiently high temperature to gel the starch throughout the pieces of vegetable and thus avoid the blanching procedure, there are other factors to be considered. Blanching destroys the enzymes and thus eliminates initial losses of vitamins during dehydration. Both carrots and sweet potatoes may lose as high as 20 per cent of their carotene in early stages of drying if not blanched. There are also other possibilities of deleterious effects upon the nutritional quality of the dried product such as have been discussed for white potatoes, Reeve (1943a).

SUMMARIZING DISCUSSION

Microscopic evidence correlated with the results of chemical analyses indicates that preservation of carotenoids (provitamin A) in certain vegetables is favored by blanching before dehydration. The objections to case-hardening effects induced by blanching of starchy vegetables do not validate rejection of the blanching process. The fact that case hardening can be remedied by thin slicing or cubing has been discussed by Reeve (1943a).

Beneficial effects of blanching are well recognized. There is less vitamin loss and the enzymes are inactivated so that certain undesirable changes are minimized. In addition, histological studies show that there is a definite correlation between blanching and the rate of carotene oxidation upon storage of the dried product, particularly in starchy vegetables. This correlation is established by comparisons of low-starch vegetables with starchy vegetables as to oil protection of the carotene and as to the protective action of gelled starch on both oils and carotene in sweet potatoes.

The use of pectin dipping of low-starch vegetables to provide a substitute for naturally occurring starch was previously suggested, Reeve (1943b). It should be re-emphasized that this method is not effective to an appreciable extent if raw slices are dipped. Blanching denatures the cytoplasm and there is a greater amount of pectin solution soaked up by blanched slices than by raw slices. Experimental treatments with pectin by dipping are further justified by the hypothesis that the pectic content of beets is of such nature as to provide a protective sealing effect in the blanched, dried tissues.

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LOSS OF ASCORBIC ACID DURING COOKING OF STORED SWEET POTATOES

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Relatively little has been reported on the vitamin C content of the sweet potato. Floyd and Fraps (1939) were the first to show that the Porto Rico variety contains an average of 20.3 mg. of ascorbic acid per 100 grams of raw peeled sweet potato and suggested that because of its extensive cultivation and use that the sweet potato might be an important source of vitamin C in the diet, particularly in Texas. Earlier, Newton and Lowry (1937) had pointed out the fact that recent dietary studies have shown that the sweet potato is a staple food among the people of the southern states as a whole. Sweet potatoes are harvested early in the fall for use throughout the ensuing year. Consequently, the purpose of this study is to determine the amount of ascorbic acid present in the raw and cooked sweet potatoes following four to six months of storage at 10°C. (50°F.) in a food-storage locker plant.

EXPERIMENTAL PROCEDURE

The eight varieties of sweet potatoes [Southern Queen, Triumph, Triumph-Purple Stem, Nancy Hall, Nancy Hall (2), Texas Porto Rico, Porto Rico (old), and Unit I Porto Rico] used in this study were furnished through the courtesy of Dr. Yarnell, Chief of the Division of Horticulture, Texas Agricultural Experiment Station.[2] All of the sweet potatoes tested were grown on the experimental station soil at Gilmer, Texas; harvested in October, 1941; and stored at approximately 10°C. (50°F.) in a food locker plant in Denton, Texas. Ten pounds of each variety were separately packaged and placed in loose wooden crates. A crate containing two varieties was removed from the locker each time and opened for testing.

The potatoes were prepared for analysis in the following ways:

Raw: All of the sweet potatoes were peeled before sampling unless otherwise stated. A slice was taken from each end and two from the center of each of four medium-sized sweet potatoes in obtaining the values reported. Using the slice method, one entire potato of the Unit I Porto Rico variety was analyzed for ascorbic acid to show the distribution throughout an individual potato. Another one of the same variety, unpeeled, was sampled in a similar manner to compare the values of the peeled with the unpeeled sweet potato.

Boiled: All but one of the eight varieties were boiled. The Porto Rico (old) variety had undergone extensive deterioration in the five months of storage and could not be cooked. A 400-gram sample of each variety was

[1] Presented in partial fulfillment of requirements for the Master's Degree.

[2] The authors are indebted to Mr. R. E. Wright, Horticulturist in charge of Sweet Potato Investigation Laboratory, Gilmer, Texas, for the selection and data pertaining to the varieties furnished.

used since this amount approximated four servings of boiled sweet potatoes. The potatoes were washed, rinsed, peeled, cut into cross sections about one-fourth of an inch thick, and dropped into 700 ml. of boiling salted water (one teaspoonful). The same covered enameled saucepan (inside diameter seven inches) was used in cooking each sample until "done." Two to three minutes elapsed before the water resumed boiling after the potatoes were added. Twelve to 25 minutes, depending upon variety, were required to boil the sweet potatoes. The water was drained from the potatoes and measured; aliquots of the cooking water were analyzed for vitamin C. The hot cooked potatoes were rapidly mixed with a wooden spoon and three samples were immediately placed in beakers containing a known amount of acid.

Candied: All but three of the varieties, Nancy Hall (2), Texas Porto Rico, and Porto Rico (old), were candied. The same procedure was used as that given under boiled except that they were boiled in a shorter time, from seven to 15 minutes. The hot slices were placed in a Pyrex baking dish and spread with two tablespoons of brown sugar and one tablespoon

TABLE 1

Ascorbic Acid Content of Peeled and Unpeeled Unit I Porto Rico Sweet Potatoes

Sample	Ascorbic acid	
	Peeled	Unpeeled
	mg./100 gm.	*mg./100 gm.*
Stem end	0.76	9.49
	1.26	......
Center	1.16	3.30
	0.72	......
	0.64	......
Root end	5.05	3.20
Average	1.59	5.

of oleomargarine for each potato and 100 ml. of the water in which the potatoes were boiled. Twenty minutes in an oven at 176.6°C.(350°F.) were required for glazing. The hot candied sweet potatoes were thoroughly mixed before three samples were removed to beakers containing a known amount of acid ready for analysis.

Baked: Four medium-sized potatoes were washed, dried, weighed, and baked for 30 to 64 minutes, depending upon the variety, in a 190.5°C. (375°F.) oven. The baked potatoes were sampled in the same manner as the raw; slices were removed from both ends and the center as soon as the potatoes were removed from the oven. These hot samples were placed in acid also.

The ascorbic acid determinations were made according to Mack and Tressler's (1936) modification of Bessey and King's (1933) method. All titrations were made in triplicate on each sample. Only averages are reported in this article.

DISCUSSION OF RESULTS

The distribution of ascorbic acid in the peeled and unpeeled Unit I Porto Rico variety sweet potato is not consistent, although all the values

for the unpeeled are higher than those for the peeled potato (Table 1). The stem-end value in the unpeeled sweet potato is almost three times higher than its root end and center, while this value is 12 times greater than the stem end of the peeled potato and nearly twice as large as the root end. Similar disagreement in the distribution of vitamin C in the Irish potato has been reported by Rolf (1940); there was a tendency for greater concentration of vitamin C to occur in the bud end although the temperature at which the tubers were stored affected this distribution. Both Wolf (1940) and Rolf (1940) conclude that the outer tissues of the Irish potato are eight to 45 per cent richer than the inner tissues. These results indicate the possibility of accidental selection of samples which would result in a relatively high or low ascorbic acid content. Consequently an average of four samples (one from either end and two from the center) were taken as the ascorbic acid value of the sweet potatoes used in this study. This method of sampling is similar to Rolf's (1940)

TABLE 2

Ascorbic Acid Content of Eight Varieties of Raw Stored Sweet Potatoes

Variety	Color	Ascorbic acid				Length of storage
		Stem end	Center	Root end	Average	
		mg./ 100 gm.	*mg./ 100 gm.*	*mg./ 100 gm.*		*mo.*
Southern Queen	White	0.59	1.05	1.83	1.14	4
Triumph	White	3.90	0.74	1.59	2.14	4
Triumph-Purple Stem	White	1.70	1.08	0.98	1.28	5
Nancy Hall (1)	Yellow	3.41	1.31	1.60	2.10	5
Nancy Hall (2)	Yellow	1.16	1.61	2.46	1.87	6
Unit I Porto Rico	Yellow	1.63	1.14	0.55	1.10	6
Texas Porto Rico	Yellow	2.09	1.19	2.40	1.56	6
Porto Rico (old)	Yellow	3.58	2.60	2.07	2.75	6

with the exception that entire sections were taken instead of wedge-shaped pieces.

Only one variety of sweet potato was analyzed for the ascorbic acid content of both the peeled and unpeeled samples. This variety, Unit I Porto Rico, had been stored for six months. Therefore, the storage temperature had been the same as for all the potatoes analyzed. That is, all of the eight varieties were stored under identical conditions, the only difference being that some remained in storage for four months and others for five and six months (Table 2). In considering the ascorbic acid content of the other varieties, there seems to be no relationship between the length of storage time and the amount of vitamin C present at the time of analysis. Porto Rico (old), which was also stored for six months, gave the highest ascorbic acid value and Southern Queen, stored only four months, gave next to the lowest value. The color of the flesh of the potato appears to be unrelated to its ascorbic acid value. Five of the eight varieties tested gave values which were higher for the stem end than for the root end. The Unit I Porto Rico values (Table 2) represent the average of four different potatoes giving a higher stem-end value but a lower average for the entire potato than is shown for the same variety (Table 1).

TABLE 3

Ascorbic Acid Losses in Cooking Eight Varieties of Sweet Potatoes

Variety and cooking method	Ascorbic acid values obtained with each variety of sweet potatoes								
	Raw	Cooked (raw basis)	Cooking water[1] serving	Loss in cooking water	Total recovery	Loss or gain in cooking	Loss or gain in cooking	Destroyed	Destroyed
	mg./ 100 gm.	*mg./ 100 gm.*	*mg.*	*pct.*	*mg.*	*mg.*	*pct.*	*mg.*	*pct.*
Southern Queen	1.14								
Boiled	......	0.17	2.40	93	2.57	1.43	126	......	....
Baked	......	0.16	......	....	0.16	—0.98	—85	0.97	85
Candied	......	0.21	2.22	91	2.43	1.29	113	......	....
Triumph	2.14								
Boiled	......	0.71	1.50	67	2.71	0.07	3	......	....
Baked	......	0.70	......	....	0.70	—1.44	—67	1.44	67
Candied	......	0.22	2.35	95	2.47	0.33	15	......	....
Triumph-Purple Stem	1.21								
Boiled	......	0.70	1.34	65	2.04	0.83	86	......	....
Baked	......	1.36	......	....	1.36	0.15	11	......	....
Candied	......	0.27	0.91	70	1.28	0.07	5	......	....
Nancy Hall (1)	1.90								
Boiled	......	0.56	6.91	92	7.47	6.57	345	......	....
Baked	......	1.87	......	....	1.87	—0.03	—0.15	0.03	0.15
Candied	......	0.61	3.50	85	4.11	2.21	116	......	....
Unit I Porto Rico	1.12								
Boiled	......	1.07	0.89	45	1.96	0.84	75	......	....
Baked	......	1.77	......	....	1.77	0.65	6	......	....
Candied	......	0.99	2.28	69	3.27	2.15	193	......	....
Texas Porto Rico	1.72								
Boiled	......	1.15	3.25	73	4.40	2.78	160	......	....
Baked	......	3.97	......	....	3.97	2.25	136	......	....
Nancy Hall (2)	1.82								
Boiled	......	1.18	2.97	71	4.15	2.33	127	......	....

[1] The potatoes were cooked in four serving lots which gave varying amounts of water. The calculations were made by apportioning the water to one serving (100 gm.) and calculating the ascorbic acid value.

The Porto Rico (old), Triumph, and Nancy Hall (1) give the highest values, namely, 2.75, 2.14, and 2.10 mg. per 100 grams of raw potato. These values are much lower than the 20.3 mg. reported by Floyd and Fraps (1939) for the Porto Rico, and the 32.6 mg. determined by Burrell and Ebright (1940) for the Jersey variety. Since factors, such as the conditions under which Irish potatoes are grown, Ijdo (1937); season and freedom from virus disease, Smith and Peterson (1937); storage time, Isumurudowa (1936); temperature of storage, Latin and Gothlin (1937); and sprouting, Kröner and Steinhoff (1937), have been shown to have an effect on the ascorbic acid content, this may account for the variations obtained in the present study. All of the stored sweet potatoes tested showed some sprouting. Latin and Gothlin (1937) and Mathiesin (1939), using the titration method of analysis for ascorbic acid, reported as much as 70 per cent lost during storage of Irish potatoes; although Woods (1935) and McKittrick and Thiessen (1932), using the biological method, found little or no change in ascorbic acid content during storage. Consequently, not only variety but the conditions of growth and storage may be assumed to affect the vitamin content of the sweet potato also. In any case, it is justifiable to assume that loss may occur during storage and for that reason not even the raw sweet potato can be relied upon to furnish vitamin C in southern diets.

The effect of boiling, baking, and candying upon the ascorbic acid content of the eight varieties of sweet potatoes studied is shown (Table 3). There was an apparent increase in the ascorbic acid content after cooking in all but three instances, namely, baked Southern Queen, Triumph, and Nancy Hall (1) sweet potatoes. Lojander (1940) reports a similar increase for cooked Irish potatoes, and attributes this in part to the difficulty of setting free the cell contents in the raw materials. Others have related the increase to the hydrolysis of a protein ascorbic acid ester during the cooking process and to the destruction of the ascorbic acid oxidase by heat. However, Rolf (1940) found that the boiling of Irish potatoes for 31 to 37 minutes caused a 10-per cent loss in ascorbic acid. This difference in results emphasizes the need for further study of the effect of cooking processes upon the ascorbic acid content of both Irish and sweet potatoes.

The ascorbic acid values for the candied potatoes includes the sugar and fat since that is the way they are consumed. The increase in ascorbic acid, five to 193 per cent, is less than that for the boiled sweet potatoes which was from three to 345 per cent. This may be attributed to a cooling of the potatoes during the transfer from the boiling water to the baking dish and the spreading of the glazing materials.

The Texas Porto Rico and Unit I Porto Rico are the only varieties showing a marked increase in ascorbic acid owing to baking; two varieties, Southern Queen and Triumph, gave markedly decreased values; while Purple Stem, Triumph, and Nancy Hall (1) gave values similar to those obtained with the raw samples. Newton and Lowry (1937) found that baked Porto Rico sweet potatoes were one-third as rich in vitamin C as raw or boiled ones. Similar variations are reported by Rolf (1940) with Irish potatoes. The latter found a loss of 15 per cent, while Richardson, Davis, and Mayfield (1937) obtained a gain of 41 per cent. The effect of

cooking processes upon the ascorbic acid content of stored sweet potatoes follows the irregularities reported for the Irish potatoes prepared in a similar manner.

The length of the cooking period varied with the variety, some potatoes requiring a longer time than others to become tender or "done." The Southern Queen, Triumph, and Purple Stem-Triumph had the whitest flesh and were drier when cooked than the yellower varieties. The length of the boiling period did not affect the resulting ascorbic acid content since two different varieties, Southern Queen and Triumph, each cooked for 12 minutes, gave .18 and .71 mg. ascorbic acid, respectively. Similar results were obtained when the sweet potatoes were candied and baked. It is evident from these data that sweet potatoes stored from four to six months at 10°C.(50°F.) and prepared by baking, boiling, and candying contribute an insignificant amount of vitamin C to the diet.

SUMMARY

Eight varieties of sweet potatoes stored from four to six months at 10°C.(50°F.) contained from 1.1 to 2.75 mg. of ascorbic acid per 100 grams of raw potato.

The ascorbic acid content was influenced more by variety than by the length of storage.

The unpeeled sweet potato contained more ascorbic acid than the peeled.

Boiled sweet potatoes contained an average of 131 per cent more ascorbic acid than the raw ones of the same variety.

The candied sweet potatoes also increased in ascorbic acid content about 108 per cent.

Baking is the only method used which showed destruction of ascorbic acid. Three of the six varieties baked showed a destruction of .15 to 85 per cent while the remaining samples showed an increase of 12 to 130 per cent.

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A MODIFIED LITTLE PLATE METHOD FOR BACTERIAL COUNTS IN VEGETABLE FREEZING PLANTS

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For many years bacterial counts have been used as a means of evaluating plant sanitation and the condition of products in food-processing establishments. The long incubation periods required for standard methods of making bacterial counts, according to Standard Methods of Water Analysis (1936), have detracted from their usefulness. Owing to the several days necessary for incubating Petri dish cultures, it has often been impossible in food plants to direct the attention of cleaning operations to those parts of the processing line which might require special care until several days after the cultures were made. For the same reason, it has not been possible, in many cases, to point out improper handling of raw materials in time to prevent processing of a considerable amount of inferior product.

Because of the need for a faster bacteriological count method, as an aid to plant sanitation in the packing of frozen foods, a number of fast-count procedures were examined. From this research a modified Frost Little Plate method was developed which has worked very well in numerous field tests.

MODIFIED LITTLE PLATE PROCEDURE

Three distinct changes were made in the Frost Little Plate Method by Tanner (1932). These changes were as follows:

1. The incubation period of four to eight hours was lengthened to 16 hours.
2. A new staining procedure was used.
3. A slide was developed which greatly facilitated the work.[1] This slide limited the culture to a definite area, prevented washing off of the agar film during staining, and allowed more thorough mixing of culture and medium. Errors, resulting from the use of a glass marking pencil to confine the culture to a definite area and caused by mixing of pencil wax and culture, were entirely eliminated by use of the new slide.

The new slide was provided with a central elevated portion, of definite area, which served as the surface supporting the culture. Slides of this type were made either by grinding away the area around the raised portion or by fusing a piece of glass, of definite area, upon a larger glass strip.

A number of the special slides were cleaned in dichromate-sulfuric acid solution, thoroughly rinsed with water, wiped dry, and sterilized with dry heat in a closed metal container.

Small batches of nutrient agar were sterilized in homeopathic vials plugged with cotton. When used, the vials were heated to melt the agar,

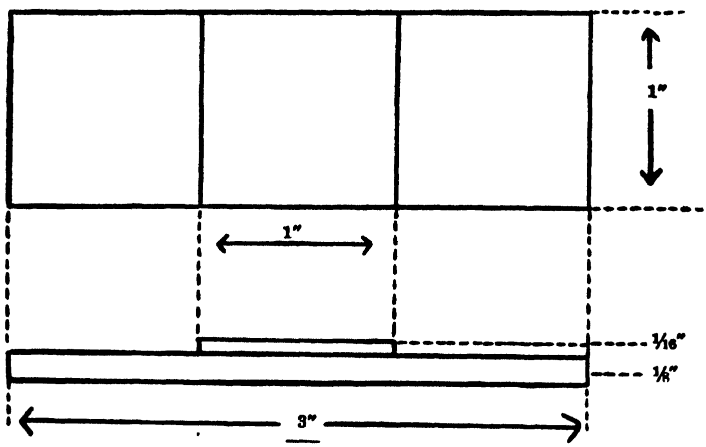
[1] The slide was developed by M. G. Hallenback, formerly with the Birds Eye Laboratories at 3 Commercial Street, Boston, Mass.

after which the cotton plug was replaced with a sterile rubber stopper fitted with a medicine dropper and bulb. During the preparation of cultures, the agar was kept molten by suspending the vials in a beaker of water kept at 45°C. (113°F.). Extension clamps were used to hold the vials.

Ten grams of the sample to be examined were placed in 90 ml. of sterile dilution water and thoroughly shaken. A sterile slide was placed on the warming table (metal plate regulated to 45°C. by clamping on a ring stand above a hot plate) and .1 ml. of the dilution water was delivered to the raised portion. The measuring pipette used (capacity 1/10 milliliter) was lined with dilution water several times by filling and emptying the pipette, and the lower portion was wiped with a sterile kleenex before the culture

SPECIAL SLIDE

PLAN



ELEVATION

sample was delivered. Four drops of molten nutrient agar (33.5 grams bacto-nutrient agar to 1,000 ml. of distilled water) were then placed on the raised portion of the slide and the material thereon mixed. Mixing was accomplished by running a sterile wire through the culture 15 times, first backwards and forwards then from right to left to right. The culture was spread to the edges of the raised portion by slanting the needle and following the edge of this area.

The slides, prepared as indicated in the foregoing, were marked for identification and placed in a moist chamber for incubation. After 16 hours' incubation at 25°C. (77°F.) cultures were removed, heated on a hotplate at about 80°C. (176°F.) until dried, treated with a one-per cent aqueous ferric sulfate solution for 20 seconds, washed, and treated with a .5-per cent aqueous solution of hematoxylin for 15 to 30 seconds. The agar film was then washed and dried.

The prepared slides were examined microscopically, using a 16-mm. objective, to detect bacterial colonies. A blood cell counter greatly facilitated this work, since it provided a means of recording both the number of fields and the number of colonies counted with a minimum of trouble.

Since .1 ml., from the equivalent of 100 ml. of dilution water, containing the bacteria from 10 grams of product, was used, the number of bacteria per gram of product was determined by the following formula:

$$100 \times \frac{\text{Area of little plate}}{\text{Area of microscopic field}} \times \frac{\text{Number of colonies found}}{\text{Number of fields counted}}$$

Since 100 fields were counted on all slides, the above formula resolved to:

$$\frac{\text{Area of little plate}}{\text{Area of microscopic field}} \times \text{Number of colonies found.}$$

RESEARCH TO EVALUATE THE MODIFIED LITTLE PLATE METHOD

In order to compare results obtained by the Modified Little Plate and Standard Petri Dish procedures, a considerable number of bacterial counts were made by both methods (A) on the same sample of frozen foods; (B) on various samples of the same kind of frozen foods taken from separate packages; and (C) on different samples of the same kind of frozen foods taken from the same package.

For the purpose of this research, different brands of frozen foods were purchased on the market. These were examined bacteriologically and samples were selected which provided material low, moderately high, and high in bacterial plate count.

A. Bacterial Counts on Same Sample of Frozen Foods (Tables 1 and 4):

Ten grams of the frozen product were weighed into 90 ml. of sterile dilution water. The bottle was shaken 300 times after which 10 .1-ml. portions of water were removed and 10 Little Plate cultures prepared as indicated in the procedure section. These cultures were incubated in a moist chamber at 25°C.(77°F.) for 16 hours then dried, stained, and counted. The original product-dilution water mixture was used to prepare Petri Dish cultures. Several higher dilutions were plated by the standard method using nutrient agar. Ten plates were made for each dilution. Petri dish cultures were incubated at 25°C. for three days, after which plates of those dilutions containing between 30 and 300 colonies were counted, Breed and Dotterrer (1916).

Bacterial counts were made as indicated on the following frozen products: broccoli, peas, green beans, and lima beans. The coefficient of variation was calculated from the results obtained with each bacterial count method for all products examined.

B. Bacterial Counts on Various Samples of Same Kind of Frozen Foods Taken From Separate Packages (Tables 2 and 4):

Duplicate bacterial counts by both Little Plate and Petri Dish procedures were made on samples taken from 12 separate packages of several frozen vegetables. The count, therefore, was determined for all samples by both methods. Cut corn, spinach, lima beans, green beans, broccoli, and peas were examined in this manner.

TABLE 1

Bacterial Counts on Same Sample of Frozen Foods[1]

Sample No.	Broccoli		Peas		Green beans		Lima beans	
	Little Plate	Petri Dish	Little Plate	Petri Dish	Little Plate	Petri Dish	Little Plate	Petri Dish
1	340,000[2]	390,000[2]	15,500	23,000	19,000	9,500	22,500	17,000
2	340,000	420,000	15,500	23,000	19,000	11,500	23,000	17,500
3	350,000	420,000	16,000	23,500	20,000	12,000	24,000	19,000
4	360,000	440,000	16,000	23,500	20,500	12,000	25,000	19,000
5	370,000	440,000	17,000	23,500	21,500	13,000	26,000	19,000
6	370,000	460,000	17,000	24,500	23,500	13,500	26,000	19,000
7	370,000	500,000	17,500	24,500	24,000	14,000	27,500	19,500
8	380,000	500,000	17,500	25,000	25,000	14,000	27,500	20,000
9	400,000	530,000	18,500	25,500	26,000	15,000	28,000	20,500
10	400,000	540,000	19,500	26,500	26,000	17,000	28,500	20,500

[1] These counts were arranged in numerical order. [2] Bacteria per gram.

TABLE 2

Bacterial Counts on Various Samples of Same Kind of Frozen Foods Taken From Separate Packages

Sample No.	Cut corn		Spinach		Lima beans	
	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]
1	350,000	380,000	71,000	56,000	18,500	22,000
2	270,000	280,000	100,000	98,000	16,500	22,000
3	270,000	320,000	580,000	520,000	22,500	26,000
4	330,000	390,000	85,000	61,000	10,500	13,500
5	450,000	460,000	140,000	110,000	37,000	31,000
6	340,000	390,000	900,000	770,000	20,500	20,000
7	260,000	250,000	85,000	55,000	17,000	24,000
8	340,000	330,000	260,000	160,000	22,500	26,000
9	240,000	300,000	270,000	150,000	11,000	14,500
10	460,000	470,000	390,000	260,000	40,500	40,000
11	180,000	210,000	420,000	420,000	42,000	41,000
12	410,000	400,000	360,000	320,000	28,500	30,000

Sample No	Green beans		Broccoli		Peas	
	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]
1	5,600	2,500	500,000	370,000	12,000	18,000
2	8,000	3,000	530,000	380,000	15,000	16,000
3	3,900	2,800	440,000	430,000	13,000	18,000
4	4,300	3,300	450,000	490,000	21,000	34,000
5	5,600	3,000	540,000	540,000	34,000	37,000
6	8,100	3,600	760,000	720,000	35,000	32,000
7	3,500	3,100	730,000	780,000	34,000	32,000
8	8,100	3,000	520,000	600,000	24,000	34,000
9	4,300	3,400	440,000	420,000	17,000	18,000
10	4,500	3,600	480,000	490,000	19,500	23,000
11	6,000	3,600	1,600,000	1,400,000	11,000	18,000
12	8,000	3,800	1,400,000	1,300,000	16,500	17,000

[1] Bacteria per gram; average of two slides. [2] Bacteria per gram; average of two plates.

TABLE 3

Bacterial Counts on Different Samples of Same Kind of Frozen Foods Taken From Same Package

Sample No.	Green beans		Lima beans		Broccoli	
	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]	Little Plate[1]	Petri Dish[2]
1	2,700	1,500	27,000	30,000	240,000	270,000
2	3,900	3,100	15,000	33,500	230,000	230,000
3	4,600	1,800	54,500	74,000	160,000	200,000
4	4,300	2,300	16,500	24,500	350,000	500,000
5	2,600	2,000	47,000	48,500	160,000	270,000
6	3,300	1,800	15,000	24,500	250,000	260,000
7	2,600	2,000	25,000	32,500	150,000	180,000
8	5,300	3,200	19,000	26,500	150,000	180,000
9	1,000	2,200	27,000	38,000	170,000	90,000
10	2,000	1,100	19,000	27,000	220,000	180,000

[1] Bacteria per gram; average of two slides. [2] Bacteria per gram; average of two plates.

TABLE 4

SUMMARY OF STATISTICAL ANALYSIS

Coefficient of Variation

Bacterial Counts on Same Sample of Frozen Foods

Sample	Modified Little Plate method	Petri Dish method
Broccoli	5.8	11.0
Peas	7.6	4.8
Green beans	13.8	15.9
Lima beans	8.0	6.0

Coefficient of Correlation

Bacterial Counts on Different Samples of Same Kind of Frozen Foods

Modified Little Plate and Petri Dish Methods

Sample	Coefficient of correlation	Analysis of significance
Cut corn	+0.95	Highly significant
Spinach	+0.87	Highly significant
Lima beans	+0.96	Highly significant
Green beans	+0.40	Not significant
Broccoli	+0.98	Highly significant
Peas	+0.86	Highly significant

Coefficient of Variation

Bacterial Counts on Different Samples of Same Kind of Frozen Foods From Same Package

Sample	Modified Little Plate method	Petri Dish method
Green beans	40.3	30.9
Lima beans	51.2	42.5
Broccoli	30.8	45.7

Correlation coefficients were calculated from the results of bacterial counts made by each method on the several different products.

C. Bacterial Counts on Different Samples of Same Kind of Frozen Foods Taken from Same Package (Tables 3 and 4):

Bacterial counts by both Little Plate and Petri Dish procedures were made on 10 different samples from the same package of frozen foods. A Modified Little Plate count and a Petri Dish count were made on each sample. Green beans, lima beans, and broccoli were examined in this manner. The coefficient of variation was calculated from the results of bacterial counts, both Petri Dish and Modified Little Plate, made by each method.

DISCUSSION

The results of bacterial counts made by standard Petri Dish and Modified Little Plate methods indicated that neither could be considered an accurate means of determining the absolute number of bacteria in frozen foods. However, they provided a sufficiently good estimate of bacterial populations to be of considerable help in evaluating the condition of food products.

Coefficients of variation calculated from bacterial counts determined by Modified Little Plate and Standard Petri Dish procedures on the same original product dilution showed that the results obtained by one method were approximately of the same precision as those obtained by the other. Bacterial counts on products containing relatively small numbers of organisms were the least precise. Since bacterial populations of such proportions are usually not of great significance in food products, this does not detract appreciably from the value of these methods of making bacterial counts.

A highly significant correlation was obtained for Petri Dish and Modified Little Plate counts on all frozen vegetables examined except green beans. In the latter case, the bacterial count was very low.

CONCLUSIONS

1. A modified Little Plate procedure for determining approximate numbers of bacteria present in food products was developed. The precision of this method was found to be of the same order as that of the Standard Petri Dish count.

2. A large variation was found in bacterial counts made on different samples from the same package of frozen foods. This was the case with both Modified Little Plate and Petri Dish methods.

3. The Modified Little Plate method, owing to the short incubation period required, should be an effective aid to the development of quality control measures in food-processing plants.

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STAPHYLOCOCCUS ENTEROTOXIN IN RELATION TO ALPHA-HEMOLYSIN PRODUCTION IN SIMPLE MEDIA

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In attempting to determine whether staphylococci suspected as the cause of food poisoning actually have the power to produce enterotoxin, the question frequently arises as to whether the medium chosen for culturing is one which may best support and develop any enterotoxigenic properties possessed by the strain under investigation. Disconcerting negative results are occasionally obtained with toxicity tests conducted with kittens, according to the procedure of Dolman and Wilson (1940), even though there is sound epidemiological evidence to incriminate a specific food product found to be heavily and significantly contaminated with staphylococci. Such findings reasonably direct attention to the possibility of incorporating in the culture medium constituents of the suspected food product. The fact that meats with a high salt content have a selective action on the growth of staphylococci has been observed by Kelly and Dack (1936). Slocum and Linden (1939) have called attention to the frequency with which the so-called ready-to-eat hams and related salt-cured meats have been involved in food-poisoning outbreaks. Pursuing the logical suggestion derived from these observations, experiments with ham have been conducted to determine its value as a basis for a medium for the identification of food-poisoning strains of staphylococci.

To obviate dependence upon maintenance of a substantial number of kittens susceptible to staphylococcus enterotoxin in order to appraise the merits of several media as substrates for toxin production, consideration has been given to measures other than animal inoculation. Favorite and McD. Hammon (1941), in the development of their casein hydrolysate vitamin medium, were guided by the generally accepted principle that enterotoxin is formed most satisfactorily under conditions which favor hemolysin production. Accordingly in these studies media composed basically of some constituent of ham were evaluated by determining the hemolysin produced in the media when inoculated with a known enterotoxic strain of staphylococci.

EXPERIMENTAL PROCEDURE

Method of Preparing Media and Cultures: Smoked ham of the "ready-to-eat" type as obtained in the market without further cooking was ground in a meat chopper and one liter of water was added to each 500 grams of ham. The mixture was allowed to stand in the refrigerator overnight. The infusion was then pressed out in a fruit press and both the pressed ham and the unfiltered infusion were used as ingredients in various media formulae. The media were sterilized in the autoclave at 15 pounds for 15 minutes, and when cool, each tube was inoculated with .5 c.c. of a broth culture of Barss enterotoxic strain, Dolman and Wilson (1938), and

then poured into a Petri dish. The inoculated media were then placed in a Brewer's anaerobic jar and supplied with an atmosphere of 30 per cent carbon dioxide and 70 per cent air. The cultures were incubated at 37°C. (98.6°F.) for 48 hours. The cultures were then centrifuged, the supernatants decanted, and the hemolysin titers determined.

The hemolysin titers obtained with various combinations of basic ham suspension, agar, and proteose peptone are shown (Table 1). For comparative purposes the table shows that a Dolman and Wilson filtrate prepared from a culture of the same strain of staphylococci used with the

TABLE 1

Hemolysin Titers

Experimental group	Formula No.	Description of media	Hemolysin titration,[1] rabbit cells	
			37°C. (98.6°F.) 1 hour	Refrigerator overnight
1	1	Basal (10 gm. infusion-free ham + 20 c.c. water)	0[2]	0
	2	Basal + .3% agar	0	0
	3	Basal + .5% proteose peptone	0	160
	4	Basal + 1% proteose peptone	40	80
	5	Basal + 2% proteose peptone	40	80
	6	Basal + .5% proteose peptone + .3% agar	40	40
	7	Basal + 1% proteose peptone + .3% agar	160	160
	8	Basal + 2% proteose peptone + .3% agar	40	40
2	9	Basal (10 gm. infusion-free ham + 20 c.c. ham infusion)	0	0
	10	Basal + .5% proteose peptone	0	0
	11	Basal + 1% proteose peptone	0	0
	12	Basal + 2% proteose peptone	0	0
	13	Basal + .5% proteose peptone + .3% agar	0	0
	14	Basal + 1% proteose peptone + .3% agar	0	0
	15	Basal + 2% proteose peptone + .3% agar	40	40
3	16	Basal (ham infusion + .3% agar)	0	0
	17	Basal + .5% proteose peptone	0	0
	18	Basal + 1% proteose peptone	0	20
	19	Basal + 2% proteose peptone	0	0
4	20	10 gm. infusion-free ham + 20 ml. Dolman and Wilson medium	20	40
	21	Dolman and Wilson medium	640[3]	640

[1] Readings are based on 50 to 100% hemolysis in the maximum dilution given. [2] No hemolysis in the lowest dilution tested, 1:20. [3] Highest dilution tested.

ham media had a hemolysin titer in excess of 1:640. It will be noted (Table 1) that infusion-free ham and ham infusion inhibit hemolysin production; furthermore, that there is a tendency for proteose peptone to stimulate hemolysin production even in the presence of ham. In no case, however, was it possible to obtain hemolysin titers in ham media comparable to the titers found with the same strains of staphylococci in Dolman and Wilson media. Attention is especially called to the extremely low titers in those media composed basically of the ham infusion. In these media even the stimulating effect of proteose-peptone is less pronounced than in the other groups. The presence also of a constituent

of the ham in Dolman and Wilson's basic media had a very inhibitory effect on hemolysin production (Group 4, Formula 20).

To determine whether the inhibition of hemolysin production could be attributed to the phenomenon of physical adsorption, a filtrate was prepared by the Dolman and Wilson method and titrations made against rabbit cells in the 37°C. water bath for one hour. Three grams of the ham were then ground with sterile sand and added to nine c.c. of the above filtrate, mixed thoroughly, and placed in a petri dish in the refrigerator overnight. After centrifugation, the alpha-hemolysin titer of the filtrates before and after adsorption was determined. No appreciable difference in titer could be demonstrated.

The low titers of hemolysin found in filtrates of the ham media noted above indicate that little value can be attached to hemolysin production as a measure for evaluation of a medium for enterotoxin production. However, it is quite possible that conditions surrounding increased hemolysin production in a simple nonprotein medium, such as the Dolman and Wilson medium, are not operative in the same manner in media composed of ham constituents.

In view of the fact that enterotoxin has been demonstrated by kitten tests in filtrates prepared from direct emulsions of ham suspected of having caused food poisoning, a simple medium consisting of chopped ham subjected to some cooking was prepared for growing suspected cultures of staphylococci to produce enterotoxin.

The smoked, but otherwise uncooked, ham was freed from the greater part of surface fat and put through a meat chopper. The freshly chopped ham was placed in Petri dishes in approximately 30-gram portions and steamed in the Arnold sterilizer for 15 to 30 minutes (autoclaving seemed to alter the ham proteins so that the emulsions subsequently prepared, because of their colloidal character, could not be filtered readily through Berkefeld candles). After cooling, the steamed ham was inoculated with the staphylococci under study, from broth cultures or other suitable suspensions of the staphylococci (veal agar slant growths suspended in saline were most commonly employed). One c.c. of inoculum was added and the contents of the dish were thoroughly mixed with a sterile spatula and then pressed down into the dish.

Preliminary experiments indicated that an artificial CO_2 atmosphere was not necessary for enterotoxin production in the chopped ham. This confirms the observations of Kelly and Dack (1936), who demonstrated in experiments with ham sandwiches that no special conditions were needed beyond an incubation long enough for production of the toxic factor. The inoculated plates were incubated, therefore, under atmospheric conditions at 37°C.(98.6°F.) for 48 hours. At the end of the incubation period the ham culture was placed in a sterile mortar and ground thoroughly, and sterile distilled water was added gradually in a known ratio. When macerated as thoroughly as possible, the emulsion was centrifuged at high speed and the supernatant fluid removed. From strongly proteolytic and lipolytic strains supernatants often were quite clear. Those with less action on the proteins and fat were colloidal. Since such materials would not readily pass through Berkefeld filters, these colloidal suspensions were

not filtered. When titration indicated that no hemolysins were present, no heating was necessary prior to the inoculation of kittens, especially when sterile material was undoubtedly used. When in doubt, a very short heating period sufficient to kill any viable staphylococci was employed, even when hemolysins were found to be absent. After heating, recentrifuging was sometimes necessary to remove precipitated material. It was noted that the hams used differed in their content of sporebearing aerobes. These, however, were not troublesome if the chopped ham, after being removed from the Arnold sterilizer and cooled, was immediately inoculated. It was found that under these conditions the staphylococci quickly outgrew these organisms.

Some preliminary results obtained on this medium with certain stock strains of staphylococci isolated during food-poisoning outbreaks suggested a number of aspects of the problem of enterotoxin production in ham which deserved investigation.

It was noted in preliminary work that the simple chopped ham medium was not only an excellent medium for the growth of staphylococci, but with certain strains enterotoxin developed in the ham medium while parallel tests, using the Dolman and Wilson medium, resulted in negative kitten reactions. This fact was notably true of three strains, isolated during food-poisoning outbreaks, with laboratory numbers 1, 6, and 40.29. In fact, when the latter strain was freshly isolated from ham involved in a food-poisoning outbreak, the test by the Dolman and Wilson method was negative, while a filtrate prepared from a chopped-ham culture of this strain was strongly positive when tested by the intraperitoneal injection of kittens. Parallel tests of Strains 1 and 6 also in preliminary trials had demonstrated enterotoxin in a ham filtrate but not in Dolman and Wilson filtrates. It therefore seemed advisable to make a comparison of Dolman and Wilson with ham media on larger series of staphylococcal strains.

Several of the strains used in this study were chosen principally because they had proven negative when tested in this laboratory, although isolated originally from food believed to be involved in a food-poisoning outbreak. This was done in order to determine whether the ham medium would bring out any latent enterotoxic potency. Strains 8A, 13B, and 17A, which were also included, had not been previously tested for enterotoxin production. They were obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, and were isolated from cases of ovine and bovine mastitis. For comparative purposes Strains 218 and 109 were included, both of which invariably produced enterotoxin by the Dolman and Wilson method. Most strains used had been carried for varying lengths of time in our laboratory culture collection on slant agar media, while some were revived from lyophile preparations.

For comparative purposes the strains were inoculated by the procedure recommended by Dolman and Wilson (1940) into their synthetic medium. Five c.c. of young tryptose broth cultures of the staphylococci were added, and incubated under 30-per cent carbon dioxide for the same incubation period employed for the ham cultures. The general technique prescribed by Dolman and Wilson was followed.

The ham filtrates were prepared by grinding the 40- to 48-hour culture ham in a sterile mortar and thoroughly macerating, in these experiments, with an equal quantity of sterile water, after which the procedure outlined above was followed.

The ham filtrates were injected into kittens intraperitoneally in 1.5-c.c. amounts up to one kilogram in weight, and increased in proportion for weights above one kilogram. In case of negative results from ham filtrates within one and one-half hours, another 1.5 c.c. was given. The additional amount did not result in increasing the positive results when the first injection was negative.

TABLE 2

Comparison of Enterotoxin Production in Chopped-Ham and Dolman and Wilson Media

Strain No.	Proteolytic action on ham	Lipolysis of fat in the ham	Appearance of the supernatant fluid	Total count staphylococci at 37°C.—48 hrs., plain agar per gram chopped ham	Titer alpha-hemolysin ham filtrate	Kitten tests, filtrates injected	
						Ham	Dolman and Wilson
1	Marked	Complete	Clear	9,200,000,000	0 [1]	+	—
6 [2]	Marked	Complete	Clear	11,000,000,000	0	—	—
11	Moderate	Complete	Clear	1,600,000,000	0	—	—
40.29	Marked	Complete	Clear	8,500,000,000	0	+	—
50 [2]	Marked	Complete	Clear	14,000,000,000	0	—	+
21	Marked	Complete	Clear	22,000,000,000	0	+	+
109	Moderate	None	Turbid	16,000,000,000	40	+	+
218	Marked	Complete	Clear	6,200,000,000	0	+	+
61	Marked	Complete	Clear	12,000,000,000	0	—	+
17A	Very slight	None	Turbid	540,000,000	0	+	—
8A	Moderate	None	Opalescent	2,300,000,000	0	+	+
13B	Moderate	None	Turbid	2,500,000,000	0	+	+

[1] Zero titer means less than 1:10. [2] On preliminary trial these strains gave positive enterotoxin on another ham medium.

The results of this comparative study (Table 2) show that eight of the 12 strains produced enterotoxin in the ham, whereas seven were positive by the Dolman and Wilson procedure. Five strains produced enterotoxin in both types of media, while three strains which were positive in the ham were negative in the Dolman and Wilson medium, and two strains were positive in the latter but not in the ham medium. It should be emphasized that the three strains which were negative in Dolman and Wilson medium continued to show negative results on retest, while of the two strains negative in ham medium one was shown to be positive by kitten test when grown on a different ham base. Furthermore, two strains, No. 11 and 6, which failed to produce enterotoxin in Dolman and Wilson or this ham medium, also were found to be positive when grown in a different basic ham medium.

Total counts of staphylococci were determined in the first series of experiments (Table 2). The first dilution was made by adding an equivalent of one gram of the ham, well emulsified as previously described in the preparation of the ham filtrates, to a dilution bottle and sufficient sterile

water was added to equal a total volume of 100 c.c. The dilution was shaken vigorously with glass beads and subsequent serial dilutions were prepared in the usual manner, plates were poured in duplicate dilutions with plain agar and incubated at 37°C. for 48 hours.

The total counts of staphylococci developing per gram in the chopped ham were all extremely high with the exception of one strain, 17A, which did not proteolyze the ham media appreciably. The count, in fact, varied greatly among those strains which apparently manifested about the same degree of proteolysis [1] but there was no strict relation of total count and enterotoxin production. Three, with counts just under 10,000,000,000 per gram, were enterotoxic, and three with counts above this figure were non-enterotoxic in the ham filtrate. In general, the total count depends greatly

TABLE 3

Preliminary Study of Effect of Different Hams on Enterotoxin Production

Ham No.	Strain No.	Proteolytic activity	Alpha hemolysin titration		Weight of kitten	Injected[1]	Reaction
			Complete	Partial			
					gm	*c.c.*	
5B	109 [2]	Poor	0 [3]	0	1,550	3.0	+
	109	Fair	0	0	850	2.0	+
	20	Marked	0	0	1,550	3.0	—
7B	109 [2]	Poor	0	0	1,100	3.0	+
	109	Poor	0	0	840	2.0	+
	20	Good	0	0	775	2.0	—
8C	109	Poor	160	....	850	2.0	—
	20	Marked	0	0	680	1.5	—
10B	109	Fair	40	80	1,200	3.0	+
	20	Marked	80	160	1,350	3.0	—

[1] Prepared in ratio of ham to water 1:1. [2] The ham medium (chopped ham in Petri dish) was autoclaved at 15 lb. for 15 min. before inoculation. [3] 0 = less than 1 to 10 dilution.

on the degree of proteolysis which results in differences in the physical dispersion of the organisms. Variations also depend on the tendency of some strains to form larger clumps than others, an observation which was confirmed by microscopic examination.

These results definitely suggested the second phase of the problem, namely, the suitability of the "ready-to-eat" or "quick-process" ham in general as a medium for testing strains of staphylococci for their ability to produce enterotoxin.

For the purposes of Experiment 3 (Table 3), four hams of different packers were inoculated with two strains of staphylococci, which in previous trials had proved to produce enterotoxin.

The effect of autoclaving the ham on enterotoxin production was determined in two hams. In the two hams so compared Strain 109 was positive

[1] The degree of proteolysis of the chopped ham was determined by observations as to the ease with which the ham formed a homogeneous emulsion when ground in the mortar with sterile water. The terms, therefore, are only roughly relative in that slight differences may not be noted.

for enterotoxin whether the ham was first autoclaved [2] or steamed before inoculation. Enterotoxin production was positive in three of the four hams with Strain 109, but Strain 20 failed to produce enterotoxin in all four hams. Apparently ham No. 8C was not suitable for enterotoxin production with either strain, but in subsequent experiments this ham did support enterotoxin production with a few strains, as will be indicated later.

To secure data on enterotoxin production of a larger number of strains of staphylococci grown in these same four hams, chopped ham media were prepared in the usual way (steamed from 15 to 20 minutes in the Arnold sterilizer) and each was inoculated with 12 known positive strains and one strain known to be negative to serve as a control; the results are tabulated (Table 4).

Enterotoxin production by the different strains grown in the four hams may be summarized as follows: with ham No. 5B, 30 per cent of the strains tested produced enterotoxin; with ham No. 8C, 33.3 per cent; with ham No. 10B, 41.6 per cent; and with ham No. 7B, 41.6 per cent. Two strains failed to develop enterotoxin in any of the hams; five strains produced toxin only once in the four hams, three twice, one three times, and one strain produced enterotoxin in all four hams.

Strain No. 61 gave a very marked entérotoxin reaction in Dolman and Wilson medium (Table 1), but in repeated trials has never shown any ability to produce enterotoxin in ham. On the other hand, Strain 40.29 has repeatedly produced enterotoxin in ham but has been negative in the Dolman and Wilson medium on a number of trials.

The results (Table 4) also demonstrate a marked difference in the ability of hams to support hemolysin production by some of the strains studied. Further, the conditions which favor an increased hemolysin production do not necessarily favor enterotoxin production. Of 18 positive enterotoxic filtrates tested, in 12 no hemolysin was produced. The remaining six positive filtrates had hemolysin titers of 1:160 or less. Attention is called particularly to some of the negative enterotoxic filtrates which disclosed larger hemolysin titers than any of the enterotoxic filtrates. Strains No. 218, 1, and 50 produced hemolysins in titers of 1:320 to 1:640. Yet these same strains, when grown in other ham media, produced enterotoxin without hemolysin production.

DISCUSSION

It has been shown in these studies that chopped ham *per se* is capable of demonstrating an enterotoxic potency with certain strains of staphylococci which fail to produce enterotoxin when grown on Dolman and Wilson medium. On the other hand, the reverse of this may be demonstrated with certain other strains of staphylococci. The Dolman and Wilson medium undoubtedly has an advantage in uniformity of composition, while chopped ham medium, although capable of demonstrating enterotoxic potency with certain negative Dolman strains, apparently differs widely in its suitability for enterotoxin production. It is questionable whether the substitution of

[2] Fifteen pounds, 20 minutes, in Petri dishes.

TABLE 4

Four Different Hams Inoculated With 12 Strains of Staphylococci

Staphylococcus strain	Ham No.	pH of filtrate	Proteolytic activity	Hemolysin titer		Enterotoxin kitten test
				Complete	Partial	
218	5B	6.50	Marked	<20	20	—
	7B	6.75	Marked	<20	<20	+
	8C	6.25	Good	<20	<20	—
	10B	7.60	Good	320	....	—
1	5B	6.40	Good	20	40	—
	7B	6.75	Marked	<20	<20	—
	8C	6.75	Good	<20	<20	—
	10B	7.50	Marked	320	640	—
11	5B	6.38	Poor	40	....	+
	7B	7.03	Fair	<20	<20	—
	8C	5.50	Poor	<20	<20	—
	10B	6.73	Fair	<20	<20	—
61	5B	6.08	Very poor	<20	<20	—
	7B	6.50	Poor	<20	<20	—
	8C	5.67	Poor	<20	<20	—
	10B	5.78	Poor	<20	<20	—
17A	5B	6.30	Poor	<20	<20	—
	7B	7.43	Fair	40	80	—
	8C	7.25	Fair	<20	<20	+
	10B	5.80	Poor	20	40	—
187	5B	6.32	Marked	<20	<20	—
	7B	7.28	Marked	80	160	—
	8C	6.74	Marked	<20	<20	—
	10B	7.57	Marked	20	40	+
21	5B	6.50	Marked	<20	<20	+
	7B	6.23	Good	40	....	—
	8C	6.73	Marked	<20	<20	+
	10B	7.23	Marked	80	160	—
50	5B	6.50	Fair	320	640	—
	7B	6.70	Marked	<20	<20	+
	8C	6.25	Good	<20	<20	—
	10B	6.42	Marked	<20	<20	—
13B Ovine mastitis	5B	6.20	Poor	<20	<20	+
	7B	8.03	Fair	<20	<20	+
	8C	5.55	Poor	<20	<20	—
	10B	8.35	Poor	<20	<20	+
8A Ovine mastitis	5B	6.36	Fair	40	....	—
	7B	7.50	Marked	80	160	—
	8C	7.27	Good	<20	<20	+
	10B	7.80	Good	80	....	+
40.29	5B	6.25	Fair	40	....	—
	7B	6.60	Marked	<20	<20	+
	8C	6.02	....	<20	<20	—
	10B	6.73	Marked	<20	<20	+
197	5B	6.53	Poor	<20	<20	+
	7B	7.40	Marked	80	....	+
	8C	7.12	Marked	<20	<20	+
	10B	7.75	Marked	80	160	+
36(209)[2] Negative strain	5B	6.3	Poor	<20	<20	—
	7B	7.3	Fair	80	160	—
	8C	7.3	Poor	<20	<20	—
	10B	6.64	Poor	<20	<20	—

[1] This strain, after repeated trials in Dolman and Wilson's medium, has never given a positive kitten test by this method. [2] This strain of staphylococci has been carried on artificial media over a period of years, and it is the strain used for antiseptic testing. It has never given a Dolman and Wilson positive test and may be considered as a definitely negative strain.

the ham medium for the Dolman and Wilson medium would serve a useful purpose, although the use of both on questionable strains might be helpful in identifying more positive strains. However, negative results with both media could not be considered conclusive evidence of the lack of ability of a strain to produce enterotoxin under certain conditions. It is quite apparent that there is no one universal medium for demonstrating the ability of some strains of staphylococci to produce enterotoxin, and further it is misleading to conclude that a particular strain is negative because it failed to produce sufficient enterotoxin in two or more different types of media to produce a positive syndrome in kittens.

Although these investigations show ham to be an excellent medium for the growth of staphylococci, very abundant growth with attendant marked proteolytic and lipolytic actions does not necessarily indicate enterotoxin production. This is true even though the strain previously produced enterotoxin in chopped-ham medium prepared from another ham manifesting more or less the same result in proteolytic and lipolytic activity. As a matter of fact, strains which do not manifest any proteolytic or lipolytic action in ham whatever may prove to be strongly enterotoxic. There appears to be no relationship between toxin production and ability of the strain studied to cause visible changes in the physical appearance of the ham. Since these studies have demonstrated the presence in ham filtrates of enterotoxin in the absence of hemolysin and hemolysin in the absence of enterotoxin, it is apparent that the conditions favoring hemolysin production bear no relationship to the mechanism of enterotoxin production.

These experiments have demonstrated that a very delicate balance exists between the organism and the medium upon which it is grown. That such is the case does not require a great deal of amplification beyond calling attention to the fact that the new process quick-cured hams, so far as we know, do not vary widely in composition, particularly those produced by the same packer. Yet the enterotoxigenic property of a particular strain of staphylococci apparently is very definitely determined, other conditions being equal, by slight differences in composition. This variation may be so slight as not to affect the ability of staphylococci to grow. Yet our results show the production of enterotoxin in the several hams is quite variable. Strains apparently vary in their sensitivity to slight variations in the composition of the ham, both in the manifestations of the degree of growth in the hams and their ability to produce enterotoxin in spite of these variations.

SUMMARY AND CONCLUSIONS

A simple chopped-ham medium for demonstrating the production of enterotoxin by staphylococci is described.

Evidence is presented to show that this medium may identify as positive certain so-called negative Dolman strains.

There is no correlation between hemolysin production and the ability of a strain to produce enterotoxin.

There appears to be no relationship between proteolytic activity of staphylococci and their ability to produce enterotoxin in ham medium.

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RELATIVE TASTE POTENCY OF SOME BASIC FOOD CONSTITUENTS AND THEIR COMPETITIVE AND COMPENSATORY ACTION[1]

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Flavor is one of the most important attributes of any food produced for human consumption. No matter how attractive the food may be in appearance, how expensively it may be packaged, or how nutritious its contents, if it does not suit the "taste" of the consumer, future sales will be negligible. In ordinary food the flavor is a mixture of true tastes and odors accompanied by a multitude of oral sensitivities. The large number of flavors associated with food are mixed in character and are influenced by touch, heat, and cold as well as odor and taste. It is unfortunate that we do not have some objective means of measuring flavor rather than the present subjective method dependent upon the vagaries of the human senses. However, considerable information has been accumulated relative to the factors affecting the flavors of foods so that it is now possible to conduct experiments in which these factors can be standardized, thereby reducing certain errors to a minimum. This has been done insofar as possible in the present work.

This work deals primarily with three basic flavors—saltiness, sourness, and sweetness—and their influence on each other. Other investigators have studied them individually as acids, salt, or sugars from various standpoints—anatomically, chemically, and physiologically—but have not studied all of them with respect to their action on each other using the same judges and technique. In food, the flavor is not dependent upon any one of the basic flavors alone. Usually a mixture of two or more are present, such as salt and sugar, acid and salt, or acid, salt, and sugar. It is, therefore, important to know the action of one on the other.

FACTORS INFLUENCING TASTE

Methods of measuring taste reactions are naturally varied, depending upon the purpose and the investigator. Some investigaters allowed the judges to sample freely the test solution, Richter and MacLean (1939) and Trout and Sharp (1937). Others have given measured amounts of the test solution, varying from five to 30 ml., King (1937), Kahlenberg (1898), and Camerer (1869); while a few placed small amounts on the protruded tongue, Shore (1892) and Biester, Weigely, and Whalin (1925).

The effect of temperature upon the taste of sapid solutions has been shown to be of considerable importance, and optimum temperatures for individual substances have been established by Hollingworth and Poffenberger (1917), Komuro (1921), Camerer (1896), Marchand (1903), and

[1] Journal Article No. 586 (n.s.).

[2] Industrial Fellow Corn Products Refining Company.

Trout and Sharp (1937). However, a few investigators have been unable to show a temperature coefficient, Kiesow (1894) and Richter and Campbell (1939).

The time interval between tasting and its effect upon sensory acuity remains uninvestigated; however, many investigators allowed specific time intervals between each tasting. Brown (1914) allowed an average of 25 seconds, Camerer (1885) used a pause from two to five minutes, and King (1937) specified a two-minute interval. Other investigators made no time specifications, allowing the judges to proceed as rapidly as desired, Trout and Sharp (1937) and Richter and MacLean (1939).

Reaction time to stimuli is a factor which has received little attention. Kiesow (1903) found the relative reaction times to be bitter > sour > sweet > saline. Hollingworth and Poffenberger (1917) showed that, with some substances, a relationship existed between temperature of the sapid solution and the reaction time.

Parker (1922) and Kahlenberg (1898) believed that distilled water exhibited real tastelessness. Titchener (1905) and Brown (1914), however, found distilled water to be most commonly described as a smooth bitter. King (1937) and Crocker and Henderson (1932) commented that it took several days of tasting before the judges became accustomed to the tastelessness of distilled water.

A number of studies have been made to determine the taste threshold of a variety of substances, but they are of little comparative value since there was no uniformity in method or definition. Titchener (1905) regarded the threshold value as that magnitude of stimulus which just brings a sensation to consciousness. Brown (1914) believed it to be midway between the intensity which is just barely strong enough to produce a sensation and the intensity which always produces a sensation. Richter and Campbell (1939) used a difference and a taste threshold. The difference threshold was the point where the judges could differentiate between a sapid solution and distilled water, while the taste threshold was the minimum concentration which the subject could recognize.

PHYSIOLOGY OF TASTE

Richards (1898), Kahlenberg (1898), and Paul (1922) believed that the sourness in acids was due to the hydrogen ion but that the intensity was due to other factors since organic acids of equal sourness had different hydrogen-ion concentrations. Crozier (1916, 1918a, 1918b) and Taylor (1927) believed that the intensity of taste was dependent upon the speed at which the acid penetrated the taste cells. Beatty and Cragg (1935) tried to correlate intensity of sourness with buffer action since they believed the sourness was related with the acid's ability to reduce the pH of the buffer. Cragg (1937a) noted that differences in the sourness of an acid could be attributed to variations in the pH of the saliva.

Saline taste, as typified by sodium chloride, can be excited by other salts to a greater or lesser degree. The chlorides, bromides, and iodides of potassium and lithium and the sulfates and nitrates of these same metals are more or less saline in taste. Kahlenberg (1898) and Hober and Kiesow (1898) showed ions to be the stimulating agent in the case of sodium

chloride. The order of effectiveness in exciting salinity was Cl > Br > I. Crozier (1934) showed colloids inactive while crystalloids in true solution were predominantly responsible for the saline taste.

Sweetness, according to Cohn (1914), was dependent upon certain structural groups, and Oertly and Meyers (1919) set up a system based upon the constitution of the chemical compound whereby they were able to predict whether a substance would be sweet or not. Finzi and Colonna (1937), in their work on the chemical constitution of sweet substances, found the theory advanced by Oertly and Meyers inadequate and cited examples of compounds that did not conform to this theory. Druce (1929) found that in a homologous series of organic compounds bitterness increased and sweetness decreased with rising molecular weight and that in many compounds the meta form was sweet while the ortho and para forms were bitter.

COMPETITION VS. COMPENSATION

Parker (1922) believed that competition rather than compensation is the rule for taste mixtures. Kiesow (1894) showed the effect of small amounts of sodium chloride in neutralizing the sweet taste of sucrose, and Zuntz (1892) showed that a one-per cent solution of sodium chloride increased the sweetness of sugar solutions. Komm and Lammer (1940) concluded that sourness of organic acids in sugar solutions is dependent upon the sugar concentration.

Cragg (1937b) concluded that sucrose decreased the sourness of hydrochloric acid and that sodium chloride had no effect. He was able to demonstrate the action of sucrose by tasting and by buffer titrations.

THRESHOLDS OF SENSATION

Fifteen judges were available throughout this part of the work. The experiments started with 25 judges but 10 were eliminated for various reasons, such as carelessness in tasting, inconsistency, and very limited availability. All the judges were students with very limited or no previous tasting experience.

The tasting was done from 100-ml. beakers, each containing five milliliters of solution which was pipetted for the judges. With each substance a judge was given a direction-data sheet. The junior author remained with the judges while tasting to insure uniform procedure.

The judges were allowed only one tasting of each solution. Each judge tasted each substance twice, at different sittings so that each threshold value represents at least 30 judgings.

The tasting work was done on basic food flavors. Calcium chloride was used, however, to determine the effect of the anion in salts upon taste by varying the cation, using mono and divalent cations.

In recording the data the first solution in the series of increasing concentration that differed from the distilled water in taste was the sensitivity threshold, whereas, the first solution in which the taste could be described was the taste threshold.

It was found that the results could be expressed best as the geometric means of the frequency distribution of the molar concentrations of the respective substances tested (Tables 1, 2, and 3). In these tables other

pertinent information is also included to facilitate interpretation of the results. The "per cent concentration" was calculated from the molar concentration. For the determination of the "relative values" the first substance listed was arbitrarily taken as 100 and the rest of the substances determined in terms of this substance by simple proportion. "Percentage" gives the relative effectiveness of the other substances in terms of the first substance listed.

DISCUSSION

Little needs to be written regarding the frequency distribution of the substances; in most cases they followed a normal distribution curve. It was intended that few or none of the judges should note any difference

TABLE 1

Relative Taste Potency of Salts

Salt	Molar concentration[1]		Per cent concentration		Relative values		Percentage	
	Sensitivity threshold	Taste threshold	Sensitivity threshold	Taste threshold	Sensitivity	Taste	Sensitivity	Taste
NaCl	.011	.039	.064	.288	1.00	1.00	100.00	100.00
$CaCl_2$	.0076	.0126	.084	.140	1.31	0.61	76.00	163.00

[1] Expressed as the geometric mean of the frequency distribution of the solutions tested.

TABLE 2

Relative Taste Potency of Acids

Acids	Molar concentration[1]		Per cent concentration		Relative values		Percentage	
	Sensitivity threshold	Taste threshold	Sensitivity threshold	Taste threshold	Sensitivity	Taste	Sensitivity	Taste
Hydrochloric	.00050	.00078	.002	.003	1.00	1.00	100.00	100.00
Lactic	.00052	.00085	.005	.008	2.50	2.67	40.00	37.45
Malic	.00043	.00075	.006	.010	3.00	3.33	33.33	30.03
Tartaric	.00041	.00070	.006	.011	3.00	3.67	33.33	27.25
Acetic	.00080	.00210	.005	.012	2.50	4.00	40.00	25.00
Citric	.00042	.00070	.008	.013	4.00	4.33	25.00	23.09

[1] Expressed as the geometric mean of the frequency distribution of the solutions tested.

between the first solution and distilled water, and usually this was the case. However, little weight was given to the exceptions since imagination and other psychological factors were undoubtedly of considerable importance.

Salts: It seems only reasonable to conclude that intensity of taste of salts rests with the particular combination of anion and cation. Sodium chloride was always described as salty. Calcium chloride was usually described as salty, but in some cases it was described as a "bitter salt" and "unknown taste." It is hard to get a group of neutral salts like NaCl to test the influence of the cation and anion on taste since many of the salts are sufficiently acid or alkaline or have some other peculiar property which makes comparison impossible. The limited work done with $CaCl_2$ and NaCl, however, would indicate that $CaCl_2$ was not as sensitive or as strong as NaCl in its taste reactions as would be indicated by the chloride-ion concentration (Table 1).

Acids: According to the results (Table 2) the order of intensity of taste of acids was HCl, lactic, malic, tartaric, acetic, citric. Taylor (1927) found the order of penetration of acids into the tissue to be HCl lactic, tartaric, malic, citric, acetic. However, where there were differences in the order, upon closer examination (Table 3) it is found that the difference is small.

COMPARISON OF TASTE AND TITRATION METHODS

Following the pattern of the work of Beatty and Cragg (1935) attempts were made to correlate sourness with titrations against a phosphate buffer, made as follows: 3.24 gm. $NaH_2PO_4 \cdot H_2O$, .48 gm. $Na_2HPO_4 \cdot 12H_2O$, 20 c.c. of approximately 1N NaOH, made up to one liter—final pH 7.05.

Ten solutions of acids ranging in molarity from .0001 to .005 (except acetic acid which ranged from .0005 to .006) were made. Ten milliliters of buffer, pH 7.05, was then titrated with each of the different molar solutions of acid until a pH of 4.45 was reached. The amount of each acid

TABLE 3

Relative Taste Potency of Sugars

Sugars	Molar concentration [1]		Per cent concentration		Relative values		Percentage	
	Sensitivity threshold	Taste threshold	Sensitivity threshold	Taste threshold	Sensitivity	Taste	Sensitivity	Taste
Sucrose	.016	.037	0.56	1.30	1.00	1.00	100.00	100.00
Dextrose	.045	.090	0.80	1.63	1.43	1.25	69.93	80.00
Fructose	.020	.052	0.35	0.94	0.63	0.72	158.73	135.14
Maltose	.038	.080	1.36	2.89	2.43	2.22	41.15	45.05
Lactose	.072	.116	2.60	4.19	4.64	3.22	21.15	31.06

[1] Expressed as the geometric mean of the frequency distribution of the solutions tested

required to change the buffer solution from pH 7.05 to 4.45 was then plotted on the vertical axis and the molar concentration of the acid used on the horizontal axis. According to Beatty and Cragg (1935) a line drawn from any point on the vertical axis parallel to the horizontal axis bisects the buffer-titration curves at equi-sour molar concentrations.

A comparison was then made of the threshold values of various acids as determined by tasting with the values found on the plotted titration curves by the method of Beatty and Cragg; the results are shown (Table 4).

The results of the two methods check remarkably well except for tartaric acid. With tartaric acid the threshold value obtained by tasting was approximately 30 per cent higher than that by titration. This exception does not invalidate the titration method but it does indicate that it should be used with caution.

Sugars: There is little that need be written about the relative taste potency of sugars; Table 3 is self-explanatory. The value for dextrose is somewhat higher than other workers have found, while the fructose value is lower. Lactose is about at the average figure, while maltose tends to be somewhat lower. Generally, the results on the sugars correspond to the range of values found in the literature. Doubtless the differences in values obtained by various investigations are due in part at least to the purity

of the sugars, especially in the case of lactose and maltose, since these are difficult to purify.

COMPETITIVE OR COMPENSATORY ACTION

The method finally chosen after trying several others consisted in matching molar solutions where the solution to be matched contained a greater than taste-threshold concentration of a substance (Column 3, Tables 5, 6, 7) and in addition, a sub-taste-threshold concentration (Column 2, Tables 5, 6, 7) but greater than sensitivity-threshold concentration of a contrasting substance. This solution was matched to a series of five solutions of increasing concentration of the greater-than-taste-threshold substance (Column 4, Tables 5, 6, 7). The series of solutions was made with sufficient difference in concentration (Column 5, Tables 5, 6, 7) so that the judges could easily notice the increase in taste. The concentrations usually varied above and below that of the solution to be matched; for example, in Table 5, .01 molar NaCl (Column 1) was added to .005 molar acetic acid (Column 2) and this solution was compared with acetic acid ranging

TABLE 4

Comparison of Threshold Values Determined by Taste and by Titration Against a Phosphate Buffer Using Hydrochloric Acid as the Standard

Acid	Molar concentration	
	Equi sour by taste	Equi sour by titration
HCl	.00078	.00078
Lactic	.00085	.00078
Malic	.00075	.00065
Tartaric	.00070	.00048
Acetic	.00210	.00230
Citric	.00070	.00062

in molar concentration from .004 to .006 (Column 4) at intervals of .0005 (Column 5). Column 6 gives the number of judges choosing the molar concentration listed in Column 7, and Column 8 the number of judges choosing the molar concentration listed in Column 9. Tables 6 and 7 follow this same general procedure except that the materials tested are listed in the respective columns of each table.

Competition or compensation was determined by the judges' choice of solution. If the substance added showed a compensatory action, it was indicated by the judges' choice of a solution other than the one of equal concentration of the substance tasted. If, on the other hand, the choice of the judges was a solution of equal concentration of the substance tasted, competition was indicated since the sub-taste-threshold substance did not add to or detract from the taste of the solution.

This method proved very satisfactory in determining the effect of sub-taste-threshold concentrations of one substance upon mildly strong tasting concentrations of a contrasting substance.

For this tasting work 100-ml. beakers were used. The judges were allowed as much solution as desired and as many retastings as they found necessary to satisfactorily choose a matched solution. It was found advis-

able to have the judges compare solutions one, three, and five with the unknown to determine the relationship and then attempt to match the unknown with a single solution by elimination. They were told that the series was in the order of increasing concentration.

Each set of solutions was tasted by 10 judges, although there were 15 available for the work, because some judges were very sensitive to either sugars or acids. Even though the sugars and acids were added in sub-taste-threshold concentration, they so altered the taste for some of the judges that matching was impossible. It was found that the 10 chosen could always make what they felt were honest matchings. Between each set of matchings, the judges were required to wait about two minutes. Part of this time was spent in thoroughly rinsing the mouth free of the preceding solution.

As a rule, sour solutions were left until the last. It was found that when they were tasted first they so sensitized the mouth that if the following series contained acids in sub-taste-threshold concentrations, the acids were tasted and in some cases persisted to such an extent that the taste of the greater-than-taste-threshold substance was almost completely obliterated.

It was also found that the judges could not do more than one series of acids at one time. Beyond this, they could not distinguish between the solutions of increasing concentration sufficiently to do satisfactory matchings. In making the sour solutions, care had to be exercised to insure that the solutions were sufficiently strong so that they could easily be tasted by all but not so strong as to be irritating to the delicate membranes of the mouth. Judges were able to taste with accuracy an average of three sets of solutions at one time with the usual two-minute rest between sets of solutions.

The influence of NaCl upon the taste of acids and sugars is given (Table 5). Sodium chloride in sub-taste-threshold concentrations consistently reduced the sourness of all the acids tested but to varying degrees. It reduced the sourness of acetic, hydrochloric, and citric acids only moderately but sufficiently for a noticeable taste difference; while with lactic, malic, and tartaric acids, sodium chloride exhibited a marked effect in reducing sourness very much greater than with the other acids. The latter three acids behaved very much alike in their taste reactions with other substances throughout the entire experiments. NaCl increased the sweetness of all the sugars tested but to varying degrees (Table 5). On the basis of concentration by weight the relative effect of NaCl on sugars was maltose > lactose > fructose > dextrose > sucrose, while on the basis of molarity it was fructose > lactose > maltose > dextrose > sucrose.

It is interesting to note that hydrochloric acid has no effect upon the taste of sodium chloride. If the intensity of taste of sodium chloride was a result of the chlorine ions, then hydrochloric acid should, if anything, increase the taste of salt by the addition of chlorine ions. Seven of 10 judges could notice no change in taste and three judges noted a reduction in taste. If the tendency is for a reduction in taste, it would seem that the sodium ion has considerable to do with the intensity of taste of salt since the sodium ions are reduced by the action of a common ion. This is also

TABLE 5

Effect of Molar Sub-Taste-Threshold Concentrations of Sodium Chloride Upon Taste of Molar Concentrations of Different Acids and Sugars

Substance	Sub-taste-threshold conc. NaCl	Sugar or acid conc.	Range in concentration	Intervals of range	Most frequent choice		Second most frequent choice	
					Number	Conc.	Number	Conc.
Acetic acid	.0100	.0050	.0040 -.0060	.0005	8	.0045	1 1	.0040 .0050
Hydrochloric acid	.0250	.0015	.0010 -.0020	.00025	7	.000125	3	.0015
Lactic acid	.020	.0030	.0010 -.0020	.00025	6	.00175	3	.0020
Citric acid	.025	.0015	.00075-.00175	.00025	7	.00125	2	.0010
Malic acid	.025	.0030	.00075-.00175	.00025	7	.00150	2	.00125
Tartaric acid	.025	.0030	.00075-.00175	.00025	5	.00150	3	.00175
Dextrose	.025	.150	.100 -.200	.025	10	.175	....	....
Sucrose	.025	.100	.050 -.150	.025	6	.125	3	.100
Maltose	.025	.125	.100 -.200	.025	9	.150	1	.175
Fructose	.025	.100	.075 -.175	.025	5	.125	4	.150
Lactose	.025	.250	.225 -.325	.025	7	.275	2	.300

TABLE 6

Effect of Molar Sub-Taste-Threshold Concentrations of Acids Upon Taste of Molar Concentrations of Sodium Chloride and Sugars

Substance	NaCl or sugar conc.	Range in conc.	Intervals of range	Most frequent choice		Second most frequent choice	
				Number	Conc.	Number	Conc.
0006 M Hydrochloric Acid							
NaCl	.100	.050-.150	.025	7	.100	3	.075
Dextrose	.175	.100-.200	.025	7	.150	3	.175
Sucrose	.100	.070-.130	.015	6	.100	2	.085
Fructose	.100	.020-.100	.020	7	.100	3	.080
.0005 M Lactic Acid							
NaCl	.100	.060-.140	.020	7	.120	3	.100
Dextrose	.175	.100-.200	.025	7	.175	2	.150
Sucrose	.115	.070-.130	.025	8	.130	2	.115
Fructose	.100	.020-.100	.020	6	.080	4	.100
.0005 M Malic Acid							
NaCl	.100	.060-.140	.020	9	.120	1	.100
Dextrose	.175	.100 .200	.025	8	.175	1 1	.150 .200
Sucrose	.100	.070-.030	.015	8	.115	1 1	.100 .130
Fructose	.100	.020-.100	.020	7	.080	3	.10
0005 M Tartaric Acid							
NaCl	.1000	.060-.140	.020	7	.120	2	.100
Dextrose	.1750	.100-.200	.025	8	.175	1 1	.150 .200
Sucrose	.1000	.070 .130	.015	6	.115	2 2	.100 .130
Fructose	.1000	.020-.100	.020	6	.080	4	.100
.002 M Acetic Acid							
NaCl	.100	.050-.150	.025	6	.125	3	.100
Dextrose	.175	.100-.200	.025	6	.150	3	.175
Sucrose	.100	.070-.150	.015	8	.100	2	.085
Fructose	.100	.020-.100	.020	7	.080	3	.100
.0005 M Citric Acid							
NaCl	.100	.060-.140	.020	7	.120	3	.100
Dextrose	.175	.100-.200	.025	8	.175	1 1	.150 .200
Sucrose	.100	.070-.130	.015	5	.115	3	.100
Fructose	.100	.020-.100	.020	7	.100	3	.080

TABLE 7

Effect of Molar Sub-Taste-Threshold Concentrations of Sugars Upon Taste of Molar Concentrations of Sodium Chloride and Acids

Substance	Sub-taste threshold	NaCl or acid conc.	Range in concentration	Intervals of range	Most frequent choice		Second most frequent choice	
	Sucrose conc.				Number	Conc.	Number	Conc.
NaCl	.030	.100	.050 -.150	.025	8	.075	2	.100
Acetic acid	.015	.0050	.0035-.0055	.0005	9	.0045	1	.0050
							2	.0006
Hydrochloric acid	.015	.0010	.0004-.0012	.0002	6	.0008	2	.0010
Lactic acid	.015	.0030	.0018-.0030	.0003	8	.0027	2	.0024
Citric acid	.015	.0010	.0014-.0010	.0002	8	.0008	2	.0010
Malic acid	.015	.0030	.0018-.0030	.0003	7	.0024	3	.0027
Tartaric acid	.015	.0030	.0018-.0030	.0003	8	.0024	2	.0027
	Dextrose conc.							
NaCl	.100	.100	.050 -.150	.025	8	.075	2	.100
Acetic acid	.075	.0050	.0035-.0065	.0005	7	.0045	3	.0050
Hydrochloric acid	.075	.0010	.0004-.0012	.0002	6	.0008	4	.0010
Lactic acid	.075	.0030	.0018-.0030	.0003	8	.0027	2	.0024
Citric acid	.075	.0010	.0004-.0012	.0002	8	.0008	2	.0010
Malic acid	.075	.0030	.0018-.0030	.0003	8	.0027	2	.0024
Tartaric acid	.075	.0030	.0018-.0030	.0003	7	.0027	3	.0024
	Fructose conc.							
NaCl	.04	.1000	.0600-.1400	.0200	8	.0800	2	.1000
							2	.0045
Acetic acid	.04	.0050	.0035-.0055	.0005	6	.0040	2	.0050
Hydrochloric acid	.04	.0010	.0004-.0012	.0002	7	.0008	2	.0010
Lactic acid	.04	.0030	.0018-.0030	.0003	9	.0027	1	.0024
Citric acid	.04	.0010	.0004-.0012	.0002	8	.0008	2	.0010
Malic acid	.04	.0030	.0018-.0030	.0003	8	.0027	2	.0024
Tartaric acid	.04	.0030	.0018-.0030	.0003	8	.0027	2	.0024
	Lactose conc.							
NaCl	.10	.100	.060 -.140	.02	6	.080	4	.100
Acetic acid	.10	.0050	.0035-.0055	.0005	6	.00045	2	.0040
Hydrochloric acid	.10	.0010	.0004-.0012	.0002	7	.0008	3	.0010
Lactic acid	.10	.0030	.0018-.0030	.0003	7	.0027	3	.0024
Citric acid	.10	.0010	.0004-.0002	.0002	6	.0008	4	.0010
Malic acid	.10	.0030	.0018-.0030	.0003	6	.0027	4	.0024
Tartaric acid	.10	.0030	.0018-.0030	.0003	6	.0024	4	.0027
	Maltose conc.							
NaCl	.06	.1000	.060 -.140	.0200	7	.080	3	.100
Acetic acid	.06	.0050	.0035-.0055	.0005	7	.0045	2	.0050
Hydrochloric acid	.06	.0010	.0004-.0012	.0002	7	.0008	3	.0010
Lactic acid	.06	.0030	.0018-.0030	.0003	8	.0027	1	.0024
Citric acid	.06	.0010	.0004-.0012	.0002	6	.0008	4	.0010
Malic acid	.06	.0030	.0018-.0030	.0003	7	.0027	3	.0024
Tartaric acid	.06	.0030	.0018-.0030	.0003	8	.0027	2	.0024

indicated in the subsequent tables where the effect of other acids upon the taste of sodium chloride is an increase in saltiness.

A study was made of the influence of NaCl on acids and sugars when the NaCl was present in sub-taste-threshold values (Table 5). The results on NaCl and sugars when the acids were present in sub-taste-threshold amounts are shown (Table 6). In these results we find that the HCl had no effect on the taste of NaCl while all the other acids increased the saltiness of NaCl. With the sugars none of the acids had any effect on the sweetness of dextrose except HCl and acetic acids which reduced the sweetness.

The sweetness of sucrose was increased by lactic, malic, citric, and tartaric and was unchanged by HCl and acetic acids. The sweetness of fructose was reduced by all the acids except HCl and citric acids in which cases it was unchanged. To check the possibility that the increase in sweetness of sucrose was due to inversion, the respective concentrations of sucrose and acids were checked with a polariscope and no inversion was found.

In the results of the influence of the sugars in sub-taste-threshold amounts upon NaCl and the acids (Table 7) a comparison of Columns 2 and 6 shows that the sugars consistently reduced the saltiness and sourness of NaCl and the acids, respectively. Sucrose reduced the sourness of malic and tartaric acids to a greater extent than any of the other sugars. There was less difference between sucrose and the other sugars in reducing the sourness of the other acids.

CORRELATION BETWEEN BUFFER TITRATION AND COMPENSATION

In the case of acids, an attempt was made to measure the change in sourness, upon the addition of NaCl and sugars, by titrating with the phosphate buffer, according to Beatty and Cragg (1935) as previously described. This was done to determine whether a relationship existed between changes in taste and buffer titration. For this purpose the buffer was made up in the same manner as before and then diluted 1 to 25. To 20 ml. of the acid, buffer was added until the solution reached pH 4.45. The amount of buffer necessary for this was recorded. This was repeated with acids to which had been added sugars or sodium chloride in the same concentration as for the previous determination. The pH of all these solutions was also taken to determine the changes, if any, in pH upon the addition of sodium chloride and sugars.

For each acid a series of seven solutions was made, the first containing only the acid and the remaining six the same concentration of acid plus the concentration of other substances (NaCl and sugars). The results of this set of determinations (Table 8) are inconsistent and are so irregular that no definite conclusions can be drawn. There was no correlation between the buffer titration and sourness as determined by taste (Table 7). It would, therefore, appear that the buffer-titration method of Beatty and Cragg would not be reliable when other substances, such as NaCl and sugars, were present. At least there was no correlation between it and the organoleptic method.

TABLE 8

Effect of Addition of Sodium Chloride and Sugars Upon Buffer Titration and pH of Acids

Molar conc. of acid	Molar conc. of substance added to acid	Ml acid necessary buffer pH 4.45	pH before buffer added
.0010 HCl	Control	21.0	3.00
.0010 HCl	.025 NaCl	20.5	3.03
.0010 HCl	.075 Dextrose	20.7	3.03
.0010 HCl	.015 Sucrose	20.6	3.05
.0010 HCl	.100 Lactose	21.3	3.02
.0010 HCl	.040 Fructose	21.0	3.06
.0010 HCl	.060 Maltose	21.0	3.01
.005 Acetic acid	Control	30.8	3.56
.005 Acetic acid	.025 NaCl	31.2	3.57
.005 Acetic acid	.075 Dextrose	30.4	3.59
.005 Acetic acid	.015 Sucrose	31.1	3.55
.005 Acetic acid	.100 Lactose	30.3	3.60
.005 Acetic acid	.040 Fructose	30.5	3.59
.005 Acetic acid	.060 Maltose	30.7	3.55
.001 Citric acid	Control	25.2	3.27
.001 Citric acid	.025 NaCl	25.5	3.30
.001 Citric acid	.075 Dextrose	25.0	3.25
.001 Citric acid	.015 Sucrose	24.8	3.28
.001 Citric acid	.100 Lactose	25.0	3.26
.001 Citric acid	.040 Fructose	25.1	3.28
.001 Citric acid	.060 Maltose	25.3	3.30
.003 Malic acid	Control	73.5	3.10
.003 Malic acid	.025 NaCl	72.0	3.05
.003 Malic acid	.075 Dextrose	73.0	3.09
.003 Malic acid	.015 Sucrose	74.0	3.10
.003 Malic acid	.100 Lactose	74.2	3.07
.003 Malic acid	.040 Fructose	73.9	3.08
.003 Malic acid	.060 Maltose	72.5	3.12
.03 Tartaric acid	Control	92.0	2.93
.03 Tartaric acid	.025 NaCl	93.2	2.97
.03 Tartaric acid	.075 Dextrose	92.8	2.95
.03 Tartaric acid	.015 Sucrose	91.5	2.91
.03 Tartaric acid	.100 Lactose	92.7	2.94
.03 Tartaric acid	.040 Fructose	93.1	2.98
.03 Tartaric acid	.060 Maltose	92.2	2.96
.003 Lactic acid	Control	60.3	3.22
.003 Lactic acid	.025 NaCl	59.5	3.20
.003 Lactic acid	.075 Dextrose	61.0	3.18
.003 Lactic acid	.015 Sucrose	61.2	3.26
.003 Lactic acid	.100 Lactose	60.8	3.22
.003 Lactic acid	.040 Fructose	59.7	3.21
.003 Lactic acid	.060 Maltose	60.9	3.24

IS THERE COMPETITION OR COMPENSATION?

The question now arises, is there competition or compensation between the three basic food flavors when two or more of them are present in a food? According to the definitions previously set up in this paper for competition and compensation in flavors, a substance was competitive when the sub-taste-threshold concentration had no effect on the contrasting substance, while it was compensatory if the sub-taste threshold added or detracted from the taste of the contrasting substance.

In the detailed effects of the acids, sugars, and sodium chloride on each other (Table 9) it will be noted, for example, that sodium chloride in

TABLE 9

Competitive or Compensatory Action of Substances Tasted

Substance tasted	Contrasting substances in sub-taste-threshold concentrations											
	Sodium chloride	Hydrochloric acid	Citric acid	Acetic acid	Lactic acid	Malic acid	Tartaric acid	Sucrose	Dextrose	Fructose	Lactose	Maltose
Sodium chloride	....	±[1]	+	+	+	+	+	—	—	—	—	—
Hydrochloric acid	....	....	....	....	....	....	....	—	—	—	—	—
Citric acid	....	....	....	....	....	....	....	—	—	—	—	—
Acetic acid	....	....	....	....	....	....	....	—	—	—	—	—
Lactic acid	....	....	....	....	....	....	....	—	—	—	—	—
Malic acid	....	....	....	....	....	....	....	—	—	—	—	—
Tartaric acid	....	....	....	....	....	....	....	—	—	—	—	—
Sucrose	+	±	+	±	+	+	+	....	....	....	....	....
Dextrose	+	—	±	—	±	±	±	....	....	....	....	....
Fructose	+	±	±	—	—	—	—	....	....	....	....	....
Maltose	+	....	....	....	....	....	....	....	....	....	....	....
Lactose	+	....	....	....	....	....	....	....	....	....	....	....

[1] ± Competitive action; + or — compensatory action.

sub-taste-threshold amounts had a compensatory action on acids and sugars since it decreased the sourness of acids and increased the sweetness of sugars. Sub-taste-threshold concentrations of acids, except hydrochloric, and sugars likewise had a compensatory action on sodium chloride since they increased and decreased the salinity, respectively. Hydrochloric acid was competitive with sodium chloride.

SUMMARY

The order of sensitivity to taste was sodium chloride > calcium chloride while the order of potency of taste was calcium chloride > sodium chloride. These differences were such that it is likely that both cation and anion play a part.

The buffer-titration method compared very favorably with the threshold taste method for determining the sourness of acids, except for tartaric acid where there was considerable difference.

The order of intensity of sourness for acids was hydrochloric acid > lactic acid > malic acid > tartaric acid > acetic acid > citric acid. Specific values for each are given.

The order of intensity of sweetness for sugars was fructose > sucrose > dextrose > maltose > lactose. Specific values for each are given.

The effect of sodium chloride was to reduce the sourness of acids and to increase the sweetness of sugars. The reduction of sourness of acids by NaCl was particularly noticeable for lactic, malic, and tartaric acids.

None of the acids had any effect on the sweetness of dextrose except hydrochloric and acetic acids which reduced the sweetness.

Acids increased the saltiness of sodium chloride, except hydrochloric acid which showed no effect.

Hydrochloric and acetic acids had no effect upon the sweetness of sucrose. The remaining acids increased its sweetness. It was found that at the concentrations used, the acids caused no inversion of the sucrose as measured by the polariscope.

The sweetness of fructose was reduced by all the acids except hydrochloric and citric acids where no change in sweetness could be noted.

All the sugars acted to reduce the saltiness of sodium chloride.

All the sugars reduced the sourness of the acids but to varying degrees. Lactic, malic, and tartaric acids were outstanding in this respect.

The effect of sodium chloride and sugars upon the sourness of acids could not be correlated with changes in phosphate buffer titrations or with changes in pH.

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EFFECT OF FERTILIZER TREATMENT ON CALCIUM, PHOSPHORUS, AND IRON CONTENT OF POTATOES [1]

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The emphasis placed on potatoes in the planning of low-cost diets increases the need for additional information concerning their nutritive value. A review of the literature reveals few studies on the variations in the mineral content and the various factors which influence the composition of this vegetable. A study was undertaken to determine the amount of calcium, phosphorus, and iron in several varieties grown under controlled conditions of soil fertilization.

The potatoes analyzed were supplied by the Division of Soils [2] from a potato-fertilizer experiment carried out during the summer of 1939. Four varieties of potato—Triumph, Early Ohio, Cobbler, and Mesaba—were grown on 14 fields of known soil type. Each field consisted of quadruplicate blocks divided into plots. The arrangement of the plots with respect to fertilizer treatment was as follows: (1) phosphate, (2) phosphate-potash, (3) untreated, and (4) nitrogen-phosphate-potash. Superphosphate (43 per cent P_2O_5) was applied at the rate of approximately 170 pounds per acre, muriate of potash (60 per cent) at 300 pounds, and sulfate of ammonia at 200 pounds. In addition, one-half of each plot was treated with iron sulfate at the rate of 25 pounds per acre.

EXPERIMENTAL PROCEDURE

Samples representative of the replicates of each field were brought into the laboratory at the time of harvesting. The potatoes were prepared for analysis by thoroughly scrubbing with a stiff brush and washing repeatedly until freed from all adhering soil. They were then washed with glass-distilled hydrochloric acid, followed by three rinsings with glass-distilled water. The potatoes were pared and cut into small pieces using a sterling silver knife. Those from the untreated plots were sampled a second time using the unpared potatoes. At least six tubers were used in the preparation of each sample, which was then air-dried to constant weight in a covered glass dish at a temperature below 90°C. (194°F.). The dried samples were ground in a glass mortar until they would pass through a fine copper sieve. Four-to-five-gram samples were ashed in triplicate in silica crucibles and analyzed for calcium and phosphorus, using the methods of Morris, Nelson, and Palmer (1931), and for iron by means of the dipyridyl method as modified by Andrews and Felt (1941). A Coleman spectrophotometer was used for the iron determinations.

Every possible precaution was taken to avoid iron contamination of the samples. In addition all equipment was treated with glass-distilled hydro-

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[2] The authors are indebted to Dr. Clayton O. Rost for supplying the potato samples.

chloric acid and only glass-distilled water was used for rinsing equipment and making solutions.

RESULTS AND DISCUSSION

Total Calcium, Phosphorus, and Iron Values: The mean values secured for calcium, phosphorus, and iron, in milligrams per 100 grams of fresh potato, (Tables 1, 2, and 3) show considerable variation in the mineral content of the potatoes grown on the various fields. The significance of these differences was tested and will be considered in succeeding paragraphs.

Effect of Field Differences: The composition of the vegetables has been reported to be influenced by a number of factors, such as variety, soil type,

TABLE 1

Calcium Content of Potatoes in Milligrams per 100 Grams Moist Weight

Field No.		Treated with P	Treated with PK	Treated with NPK	Not treated with P, PK, NPK		Variety	Soil type
		Pared			Not pared			
1	Iron sulfate	7.7	7.7	7.5	8.1	9.2	Early Ohio	Bearden silt loam
	No iron sulfate	7.7	6.9	7.1	8.0	9.0		
12	Iron sulfate	7.6	9.3	8.5	8.3	9.6	Early Ohio	Bearden silt loam
	No iron sulfate	7.9	7.3	7.4	6.8	8.8		
2	Iron sulfate	7.6	9.0	7.0	7.8	8.0	Cobbler	Bearden silt loam
	No iron sulfate	7.9	7.3	6.5	7.2	10.6		
11	Iron sulfate	5.4	5.3	5.8	6.4	7.7	Cobbler	Bearden silt loam
	No iron sulfate	6.5	8.3	6.8	5.7	7.3		
10	Iron sulfate	6.9	4.5	4.6	4.1	5.6	Cobbler	Ulen loamy sand
	No iron sulfate	5.7	5.2	6.6	5.9	7.8		
13	Iron sulfate	4.4	4.8	5.3	4.4	6.6	Cobbler	Ulen loamy sand
	No iron sulfate	4.9	5.0	4.8	5.0	6.3		
5	Iron sulfate	5.7	7.4	7.0	6.8	9.1	Cobbler	Fargo silty clay loam
	No iron sulfate	7.3	7.5	7.0	8.5	8.6		
7	Iron sulfate	5.6	7.1	5.5	6.7	9.0	Cobbler	Fargo silty clay loam
	No iron sulfate	6.4	6.2	6.7	6.9	8.1		
14	Iron sulfate	7.6	9.1	7.5	7.7	13.0	Triumph	Bearden loam
	No iron sulfate	8.1	9.0	8.7	9.0	14.7		
15	Iron sulfate	6.5	6.1	6.3	6.2	9.7	Triumph	Bearden loam
	No iron sulfate	5.7	6.7	6.1	6.4	8.3		
3	Iron sulfate	6.4	6.6	8.3	6.6	10.7	Triumph	Fargo silty clay loam
	No iron sulfate	7.1	7.9	7.7	7.8	10.8		
4	Iron sulfate	7.2	7.8	8.7	7.6	13.6	Triumph	Fargo silty clay loam
	No iron sulfate	9.0	7.3	9.1	8.4	8.9		
6	Iron sulfate	5.3	6.7	7.4	7.4	8.5	Triumph	Fargo silty clay loam
	No iron sulfate	6.0	8.8	7.1	7.5	9.3		
8	Iron sulfate	5.4	4.3	4.8	4.8	5.7	Mesaba	Fargo silty clay loam
	No iron sulfate	4.9	5.2	4.3	4.8	5.5		
Mean—iron sulfate		6.4	6.8	6.7	6.6	9.0		
Mean—no iron sulfate		6.8	7.0	6.8	7.0	8.9		

climate, season, fertilizer treatment, etc. In order to investigate the effect of field differences and of fertilizer treatments on the calcium, phosphorus, and iron content, analyses of variance were computed on the accumulated data (Tables 4, 5, and 6). It is evident from these tables that the diversity in calcium, phosphorus, and iron values attributable to the fields on which the potatoes were grown was highly significant. That these variations are associated with field differences rather than with variety of potato or soil type is apparent (Tables 1, 2, and 3). It may be observed that marked divergence in mineral content occurred even when the same variety of potato was grown on fields of the same type of soil. Many of these differ-

TABLE 2

Phosphorus Content of Potatoes in Milligrams per 100 Grams Moist Weight

Field No.		Treated with P (Pared)	Treated with PK (Pared)	Treated with NPK (Pared)	Not treated with P, PK, NPK (Not pared)		Variety	Soil type
1	Iron sulfate	43.0	34.8	35.3	33.3	31.7	Early Ohio	Bearden silt loam
	No iron sulfate	34.4	34.8	38.1	34.8	33.4		
12	Iron sulfate	46.3	42.9	43.9	41.4	40.0	Early Ohio	Bearden silt loam
	No iron sulfate	47.9	42.0	43.7	41.5	38.6		
2	Iron sulfate	55.7	55.5	54.8	56.1	55.1	Cobbler	Bearden silt loam
	No iron sulfate	58.5	51.0	59.9	55.8	55.8		
11	Iron sulfate	59.8	58.6	64.1	59.0	50.9	Cobbler	Bearden silt loam
	No iron sulfate	63.1	59.5	60.8	50.9	51.4		
10	Iron sulfate	59.4	46.1	51.2	53.5	49.7	Cobbler	Ulen loamy sand
	No iron sulfate	58.5	50.0	55.2	53.8	50.7		
13	Iron sulfate	43.4	40.2	43.8	43.3	40.7	Cobbler	Ulen loamy sand
	No iron sulfate	42.1	38.2	42.0	44.7	43.3		
5	Iron sulfate	68.0	74.8	71.9	73.0	74.0	Cobbler	Fargo silty clay loam
	No iron sulfate	77.2	84.4	83.4	80.6	72.4		
7	Iron sulfate	54.4	53.0	51.3	48.1	46.8	Cobbler	Fargo silty clay loam
	No iron sulfate	56.7	57.9	51.9	51.5	46.7		
14	Iron sulfate	80.6	81.7	75.9	82.5	79.1	Triumph	Bearden loam
	No iron sulfate	87.6	74.8	70.8	77.5	78.8		
15	Iron sulfate	56.0	44.1	44.0	46.6	45.7	Triumph	Bearden loam
	No iron sulfate	51.0	47.3	48.0	44.3	42.9		
3	Iron sulfate	54.6	65.0	62.9	76.9	55.9	Triumph	Fargo silty clay loam
	No iron sulfate	67.9	57.7	55.7	56.9	50.6		
4	Iron sulfate	75.6	69.2	74.5	81.3	76.9	Triumph	Fargo silty clay loam
	No iron sulfate	83.3	76.3	72.3	77.3	76.2		
6	Iron sulfate	52.2	49.4	47.9	48.2	39.5	Triumph	Fargo silty clay loam
	No iron sulfate	57.3	55.8	43.6	46.8	45.7		
8	Iron sulfate	73.4	73.0	67.8	69.8	64.4	Mesaba	Fargo silty clay loam
	No iron sulfate	74.0	76.4	73.7	62.5	58.8		
Mean—iron sulfate		58.7	56.3	56.4	58.1	53.6		
Mean—no iron sulfate		61.5	57.6	57.1	55.6	53.2		

ences were found to be statistically significant; for example, Triumph potatoes grown on two fields[3] of Bearden loam contained significantly different quantities of calcium ($t = 7.87$, $P = <.001$), phosphorus ($t = 13.24$, $P = <.001$), and iron ($t = 14.33$, $P = <.001$). Similarly, significant differences in calcium ($t = 2.20$, $P = .043$) and phosphorus ($t = 10.09$, $P = <.001$) but not in iron values were secured for Cobbler potatoes grown on two fields[4] of Fargo silty clay loam.

TABLE 3

Iron Content of Potatoes in Milligrams per 100 Grams Moist Weight

Field No.		Treated with P	Treated with PK	Treated with NPK	Not treated with P, PK, NPK		Variety	Soil type
		Pared			Not pared			
1	Iron sulfate	.30	.30	.26	.27	....	Early Ohio	Bearden silt loam
	No iron sulfate	.27	.33	.32	.28	.33		
12	Iron sulfate	.30	.24	.31	.27	.28	Early Ohio	Bearden silt loam
	No iron sulfate	.29	.30	.29	.27	.29		
2	Iron sulfate	.40	.38	.42	.36	.46	Cobbler	Bearden silt loam
	No iron sulfate	.45	.38	.43	.39	.46		
11	Iron sulfate	.39	.42	.40	.41	.38	Cobbler	Bearden silt loam
	No iron sulfate	.39	.43	.44	.37	.38		
10	Iron sulfate	.34	.33	.34	.35	.37	Cobbler	Ulen loamy sand
	No iron sulfate	.38	.36	.41	.37	.42		
13	Iron sulfate	.29	.29	.31	.32	.31	Cobbler	Ulen loamy sand
	No iron sulfate	.30	.34	.31	.31	.36		
5	Iron sulfate	.40	.43	.42	.46	.48	Cobbler	Fargo silty clay loam
	No iron sulfate	.49	.50	.51	.46	.48		
7	Iron sulfate	.51	.47	.43	.47	.54	Cobbler	Fargo silty clay loam
	No iron sulfate	.51	.52	.42	.48	.61		
14	Iron sulfate	.54	.50	.50	.52	.53	Triumph	Bearden loam
	No iron sulfate	.49	.47	.46	.55	.56		
15	Iron sulfate	.34	.30	.31	.33	.38	Triumph	Bearden loam
	No iron sulfate	.32	.32	.33	.28	.31		
3	Iron sulfate	.42	.46	.43	.40	.45	Triumph	Fargo silty clay loam
	No iron sulfate	.45	.38	.44	.38	.41		
4	Iron sulfate	.49	.44	.46	.47	.51	Triumph	Fargo silty clay loam
	No iron sulfate	.61	.56	.50	.47	.55		
6	Iron sulfate	.39	.40	.39	.42	.42	Triumph	Fargo silty clay loam
	No iron sulfate	.42	.40	.42	.42	.51		
8	Iron sulfate	.42	.44	.41	.39	.42	Mesaba	Fargo silty clay loam
	No iron sulfate	.41	.42	.42	.34	.38		
Mean—iron sulfate		.40	.39	.38	.39	.43		
Mean—no iron sulfate		.41	.41	.41	.38	.43		

[3] Fields 14 and 15.

[4] Fields 5 and 7.

TABLE 4

Analysis of Variance of Calcium Values of Pared Potatoes in Soil-Treatment Experiment

Source of variation	Degrees of freedom	Sum of squares	Mean square
Fields	13	138.98	10.69 [1]
Treatments (P, PK, NPK, O [2])	3	1.80	0.60
Error (a)	39	18.76	0.48
Iron	1	2.11	2.11 [3]
Iron x treatments	3	0.39	0.13
Iron x fields	13	10.24	0.79
Error (b)	39	17.84	0.46
Total	111	190.12	....

[1] Highly significant. [2] O indicates not treated with P, PK, NPK. [3] Significant.

TABLE 5

Analysis of Variance of Phosphorus Values of Pared Potatoes in Soil-Treatment Experiment

Source of variation	Degrees of freedom	Sum of squares	Mean square
Fields	13	19,719.75	1,516.90 [1]
Treatments (P, PK, NPK, O [2])	3	219.22	73.07 [3]
Error (a)	39	738.55	18.94
Iron	1	8.36	8.36
Iron x treatments	3	97.08	32.36
Iron x fields	13	299.43	23.03
Error (b)	39	537.65	13.79
Total	111	21,620.04	....

[1] Highly significant. [2] O indicates not treated with P, PK, NPK. [3] Significant.

TABLE 6

Analysis of Variance of Iron Values of Pared Potatoes in Soil-Treatment Experiment

Source of variation	Degrees of freedom	Sum of squares	Mean square
Fields	13	.5807	.0447 [1]
Treatments (P, PK, NPK, O [2])	3	.0045	.0015
Error (a)	39	.0360	.0009
Iron	1	.0057	.0057 [1]
Iron x treatments	3	.0036	.0012
Iron x fields	13	.0201	.0015
Error (b)	39	.0223	.0006
Total	111	.6729	....

[1] Highly significant. [2] O indicates not treated with P, PK, NPK.

Effect of Fertilizer Treatments: The analysis of variance indicated, further, that treatment with phosphate, phosphate-potash, or nitrogen-phosphate-potash was without influence on the calcium values of the pared potatoes. However, application of iron sulfate resulted in a significant difference in the content of this mineral. A comparison of the means (Table 1) shows that a decrease in calcium of 4.3 per cent or .3 milligram per 100 grams of pared potato resulted from the iron-sulfate treatment.

A significant difference in phosphorus values owing to fertilizer treatment was disclosed by means of the analysis of variance. To ascertain which fertilizer treatments were the causative factors, it was necessary to eliminate the effect of field differences by basing the comparisons on data secured from the same field. For these computations the standard error of the mean difference was used for determining whether the phosphorus values for potatoes grown on fertilizer-treated plots differed significantly from those grown on the untreated plots of the same field. Separate calculations were made for phosphate, phosphate-potash, and nitrogen-phosphate-potash treatment, with and without iron sulfate, as compared with the untreated plots. The results demonstrated that the phosphorus values of the potatoes grown on the plots to which phosphorus alone had been added were significantly greater than those for the potatoes from the untreated plots ($t = 4.17$, $P = <.001$). The mean increase observed was 10.4 per cent or 5.8 milligrams per 100 grams of potato. Since potassium was added to the other fertilized plots, it would appear that this element suppressed phosphorus utilization. Furthermore, iron sulfate would also appear to be an inhibiting factor since a nonsignificant increase in phosphorus content was noted when this compound was added to the phosphorus-treated plots.

The fertilizer treatments were without effect on the iron values. Application of iron sulfate to the fields resulted in a significant difference in the iron content of the potatoes. It is apparent that potatoes grown on the plots to which iron sulfate had been added contained less iron than did those from plots not so treated (Table 3).

Elmendorf and Pierce (1940) studied the effect of varying amounts of nitrogen, phosphorus, and potassium in fertilizer on the calcium and phosphorus content of four vegetables—beets, cabbage, corn, and beans. They found that all fertilizer-treated vegetables contained more phosphorus. Two of the vegetables, corn and beans, when grown on fertilized soils contained less calcium than when grown on unfertilized soils. They suggest that the calcium in the vegetable can be replaced by another base, possibly potassium, which is supplied from the fertilizer. Bishop (1934) reported that with increasing rates of super-phosphate treatment regular increases in the phosphorus content of vegetables were noted, but the calcium changes were small. Coleman and Ruprecht (1935), on the other hand, found very little influence on the composition of crops as a result of the addition of complete fertilizer to the soil.

Relationship Between Calcium, Phosphorus, and Iron Values: The relatively wide variations noted in the content of calcium, phosphorus, and iron suggested the desirability of determining whether high values for one element were paralleled by correspondingly high values for the other

elements. The coefficients of correlation were calculated between the several pairs of variates using the 56 values for pared potatoes from plots to which no iron sulfate had been applied and, similarly, from those treated with this compound (Tables 1, 2, and 3). The results of these computations showed a high degree of relationship between the phosphorus and iron values of the potatoes both when no iron sulfate had been added to the soil ($r = .81$, $P = <.001$) and when it had been applied ($r = .80$, $P = <.001$). Calcium and iron values for the potatoes from plots not treated with iron sulfate were also significantly correlated ($r = .40$, $P = >.001$). However, as might be anticipated from previous discussion of the effect of application of iron sulfate on the calcium and iron values, no relationship was noted between these minerals in potatoes from plots so treated. A significant relationship was also observed between the calcium and phosphorus values from plots without iron sulfate ($r = .30$, $P = <.021$), but no relationship between these elements was demonstrated for the potatoes from the treated plots.

Effect of Paring: Losses in the nutritive value of potatoes as a result of paring have been frequently emphasized. In order to establish the extent of these losses, the differences in the calcium, phosphorus, and iron values of pared and whole potatoes were investigated on samples from those grown on the untreated plots. The results of these comparisons showed that the pared samples contained a significantly smaller amount of calcium and of iron than did the unpared samples. The decrease in the calcium content of the potatoes was 24 per cent or 2.1 milligrams per 100 grams ($t = 7.09$, $P = <.001$), while that for the iron was 10.5 per cent or .04 milligram per 100 grams ($t = 5.66$, $P = <.001$). Conversely, a significantly higher value for phosphorus was obtained for the pared potatoes when contrasted with the unpared samples. The mean difference was 6.5 per cent or 3.5 milligrams per 100 grams of potato ($t = 4.24$, $P = <.002$). These results indicate that the calcium and iron are more highly concentrated in the skin and cortical layer while the phosphorus is in the medulla of the potato.

SUMMARY

The effect of fertilizer treatments on the calcium, phosphorus, and iron values of four varieties of potato grown on 14 fields of known soil type was investigated. The arrangement of the plots in each field with respect to treatments was as follows: (1) phosphate, (2) phosphate-potash, (3) untreated, and (4) nitrogen-phosphate-potash; in addition iron sulfate was applied to one-half of each plot.

Factors inherent in the soil of the various fields were apparently of primary importance in determining the calcium, phosphorus, and iron content of the potatoes, as shown by the highly significant differences which were attributable to the fields.

The phosphate, phosphate-potash, and nitrogen-phosphate-potash treatments had no significant influence on the calcium, phosphorus, and iron content of the pared potatoes, with the exception of the phosphorus values from the plots to which phosphorus alone had been added. These showed a mean increase in this element of 10.4 per cent as compared with the untreated plots. Since potassium or iron sulfate or both had been added to

the other plots, these results would indicate that these applications suppressed phosphorus utilization.

A statistically significant decrease in the calcium values of the potatoes resulted from the iron-sulfate treatment. The mean decrease noted was 4.3 per cent.

The potatoes from the sulfate-treated plots contained a significantly smaller amount of iron than did those from the plots to which no iron sulfate had been applied.

A highly significant positive correlation was observed between the phosphorus and iron values for the potatoes grown on the iron sulfate-treated plots as well as those from the plots to which no iron sulfate had been added. Calcium and iron values for the potatoes from the untreated plots were positively correlated, as were also calcium and phosphorus; but no significant relationship was observed between these values for the potatoes from plots treated with iron sulfate.

Paring potatoes resulted in significant losses in the total calcium and iron of the vegetable, 24 and 10.5 per cent, respectively, indicating that these elements are more highly concentrated in the skin and cortical layer. The phosphorus would appear to be concentrated in the medulla of the tuber since significantly higher values, 6.5 per cent, were obtained for the pared than for the unpared samples.

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PALATABILITY STUDIES ON POULTRY: A COMPARISON OF THREE METHODS FOR HANDLING POULTRY PRIOR TO EVISCERATION[1]

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The complete preparation of poultry (dressing, evisceration, disjointing, and packaging) has shown remarkable progress in the past ten years. Much of this progress has occurred in the Middle West. The factory practices have become quite well standardized into rapid, continuous line operation, as reported by Stewart and Drews (1938) and Finnegan (1941). The handling of as many as a thousand birds an hour is now quite common. This mechanization of the ready-to-cook poultry preparation process involves a considerable capital overhead and also requires the training of semiskilled workers. The movement of poultry from the farm to the poultry packing plant in the Middle West is highly seasonal. Over 50 per cent of the annual total is marketed during the months of September, October. November, and January, Oderkirk (1939). Both of these factors influence, to a considerable extent, the total cost of preparing eviscerated poultry for market. So long as it is necessary to process completely all poultry during the season of heavy marketing, approximately four months, the overhead cost will continue to be high.

To relieve this situation, it has been suggested that the poultry might be stored New York dressed. The thawing, eviscerating, disjointing, and packaging of the birds could then be done over an extended period of time. If this procedure could be followed without appreciably affecting quality or increasing costs, the equipment overhead could be maintained at a comparatively low figure. Also by using workers over the longer period of time each year more skill could be developed and less labor turnover would probably result.

Lowe (1939) reported a study which bears on this matter. Two series of New York dressed birds were frozen three hours and 18 hours after slaughtering. The birds were then stored for varying lengths of time before thawing and evisceration. The abdominal cavity aromas of the uncooked birds were less desirable in those stored for 90 days before eviscerating.

Since this work was reported the authors have reported on other detailed studies of the post-mortem changes which occur in undrawn poultry, Stewart, Lowe, and Morr (1941) and Hanson, Stewart, and Lowe (1942). As a result it seemed desirable to reinvestigate certain phases of the original problem. The location and kind of off-flavors in dressed poultry are now better understood, and recognition of the organoleptic changes occurring during processing and storage are now much more precise.

It was felt that comparisons should include fresh controls, that is,

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birds killed, eviscerated, and held just long enough to become tender. Such controls, code marked to prevent visual identification, serve to establish a relatively constant base line for the comparisons being made. Results of many tests indicate that, except for tenderness and perhaps in cases of abnormal flavors owing to diet, these fresh controls are superior in eating quality to birds which have been treated further, i.e., held, frozen, etc.

In the present study a comparison was made of three commercial methods of handling poultry prior to eviscerating. They were (1) poultry chilled for 18 hours, then eviscerated, disjointed, packaged, and quick-frozen; (2) New York dressed poultry chilled, frozen in a brine spray, stored for approximately 30, 60, 90, and 120 days, thawed, eviscerated, disjointed, packaged, and quick-frozen; (3) New York dressed poultry chilled for 18 hours, packed, frozen in air, stored for approximately 30, 60, 90, and 120 days, thawed, eviscerated disjointed, packaged, and quick-frozen; and (4) poultry eviscerated and disjointed immediately after dressing, then chilled for 15 to 23 hours before cooking (fresh controls).

EXPERIMENTAL PROCEDURE

A total of 100 frying chickens (2½ to 3¼ pounds New York dressed) was used in these experiments. Twenty were fresh controls, 70 had been stored for six months, and the remaining 10 birds had been stored for 12 months. With all birds, except the controls, the dressing, chilling, and brine-spray (Z) freezing were done in a large produce plant in Sioux City, Iowa. The air and multiple-plate (Birdseye) freezing and the eviscerating and disjointing were done in a large eviscerating plant in Omaha, Nebraska. The birds were transported from Sioux City to Omaha (100 miles) in a refrigerated truck.

All of the birds were selected for uniformity of character and kind immediately after plucking. The fresh controls were selected, after dressing, from birds of the Experiment Station flock. In quality and kind they were as nearly like the birds originally selected as was possible. All birds were semi-scalded and machine picked. All except the fresh controls were also wax finished. Each bird was tagged with a wing band carrying a code number.

The following treatments were compared:

1. *Fresh Controls:* The birds were eviscerated and disjointed promptly after dressing, then cooled and held in air at 1.7°C.(35°F.) for 15 to 23 hours before cooking and scoring. One such bird was used every time comparisons were made.

2. *Sharp-Frozen Series:* The birds were chilled in ice water for two hours. There was no actual control of the water temperature though a great excess of ice was always present. They were then removed, packed in a clean sugar barrel with a great excess of cracked ice, held for 16 hours (including refrigerated truck transport to Omaha). The birds were then removed [internal temperature .6 to 1.1°C.(33 to 34°F.)], packed in poultry boxes lined with a moisture-vapor-resistant paper (unbranded sample, Westfield River Paper Company, Russell, Mass.), frozen, and

stored in air at —20.6°C.(—5°F.). Five birds at a time were drawn from this lot after storing approximately 30, 60, 90, and 120 days and 10 birds after 180 days. They were thawed in running water at 15.6 to 21.1°C. (60 to 70°F.); three to three and one-half hours were required. They were then eviscerated and disjointed, packed in individual cartons lined with moisture-vapor-resistant sheets (M.A.T. cellophane), code marked, and frozen in a multiple-plate freezer at —33.3 to —34.4°C.(—28 to —30°F.) (Birdseye), then returned to the storage room for the balance of the holding period.

3. *Brine-Spray ("Z" Process) Series:* Birds were handled as under the second treatment until removed from the ice water. They were then packed in frames, frozen in the brine spray at —20.6°C.(—5°F.) for two and one-half hours, ice glazed, packed in wax paper-lined (30- to 40-pound stock) poultry boxes, and finally placed in a freezer at —17.8°C. (0°F.) until shipment to Omaha. They were shipped as described under Treatment 2. On arrival they were placed in a room at —5°F. Samples were removed and preparation completed exactly as previously described after approximately 30, 60, 90, 120, and 180 days.

4. *Chilled Series:* The birds in this series were treated exactly as in Treatment 2 until they reached Omaha. They were then promptly eviscerated, disjointed, packaged, multiple-plate frozen, and stored as described in the latter part of Treatment 2.

At the end of the six-months' storage period the five birds in each series were removed, thawed in an electric refrigerator at 4.4 to 7.2°C. (40 to 45°F.) for 48 hours, and prepared for the organoleptic tests. The remaining 15 birds (five from the chilled series and five each from the 180-day period of both the sharp-frozen and brine-spray frozen series) were stored for an additional six months and then examined.

The birds in the various lots were randomized so that every treatment was compared with every other treatment. A fresh control was included in every comparison, but it was code marked and the judges had to rely solely on their organoleptic senses to detect its presence. This they did with remarkable accuracy.

Appearance and Aroma Scores: After the final thawing one of the authors scored the poultry from each of the treatments for aroma of the kidney area, giblets, general abdominal area, and vapors escaping when the oven was opened for determining the completion of cooking (10 equalled perfect; 0 equalled extremely unpalatable). Other observations were made on general physical appearance of the poultry during their preparation and cooking.

Palatability Scores: The disjointed chicken (except the giblets) of each sample was cooked on a rack in an uncovered roasting pan in a gas oven at 162.8°C.(325°F.) until considered done. The giblets were cooked in a double boiler; the heart and gizzard were cooked in 120 c.c.. of water for 25 minutes, then the liver was added and all were cooked for 20 minutes more.

Pieces of the thigh, breast, liver, and gizzard were scored by the three authors for flavor, juiciness, and tenderness, using a 10-point scoring range

(10 equalled perfect; 0 equalled extremely unpalatable). Practically all the samples were scored immediately after cooking, i.e., they were still warm.

The significance of all scores was determined by analysis of variance.

GENERAL RESULTS

At the time the birds were eviscerated it was observed that practically all livers in the birds of the chilled and sharp-frozen series showed gall stains. In only two instances, however, did the birds in the brine-spray series show this defect. Most of the livers from the birds which had been frozen appeared quite soft and mushy while the others were tough and hard when cooked. Many of them did not have the normal greyish-red color after cooking but were usually yellow (sometimes very intense) or brown. Somewhat similar observations were reported recently by Kiermeier (1939).

TABLE 1

Effect of Storage Period Before Eviscerating on Uncooked Liver Aroma Scores

(Six Months' Storage)

Days in storage before thawing and eviscerating (approximate)	Number of birds	Scores — Method of freezing: Brine-spray	Scores — Method of freezing: Sharp-frozen
30	5	8.0	5.6
60	5	8.6	6.2
90	5	7.6	7.4
120	5	7.2	6.0
180	5	7.6	5.8

There was practically no evidence of desiccation (loss of bloom, pock marking, or freezer burn) on any of the birds. The ice glaze and the moisture-vapor-resistant wrappings proved to be adequate protection under the conditions encountered in these experiments.

There were no significant differences in the amount of drip found in the cartons following defrosting between the birds twice frozen (the brine-spray and sharp-frozen series) and those once frozen (chilled series).

Storage Period Before Evisceration: The average scores for the uncooked liver aroma for the various storage periods with the two different methods of freezing the New York dressed birds (Table 1) show no significant difference in the aroma scores that can be attributed to the length of the storage period. The remainder of the results involving storage time before eviscerating are not given since no differences were found. In other words, the length of the storage period before evisceration did not influence the palatability scores. The differences between the two methods of freezing are significant, however.

Method of Handling Prior to Evisceration: The average scores for each factor, the difference between that score and those of each of the other treatments, and the significance of these differences are given (Tables 2A, 2B, 3A, and 3B).

It will be recalled that a much more extensive comparison was made of the brine-spray and sharp-freezing methods after varying intervals of storage and the fresh controls. These data are presented for each factor separately.

DISCUSSION

Six Months' Storage Period: The aroma scores of the uncooked birds show that, in general, none of the treatments yielded values quite as high as those for the fresh controls. The birds of the brine-spray series were consistently better than those of the chilled series, which, in turn, were better than those of the sharp-frozen series. The differences here were not significant except in the extended comparison of the brine-spray and sharp-frozen series (all storage periods). In this case, the birds in the brine-spray series were significantly superior to those in the sharp-frozen series.

TABLE 2A

Aroma Scores of Poultry Handled in Various Ways

(Six Months' Storage)

Treatment	All methods			Freezing methods		
	Number of birds	Average score	Difference	Number of birds	Average score	Difference
1. *Kidney area*						
Fresh	5	9.8	....	25	9.2	....
vs. brine-spray	5	8.6	1.2	25	8.1	1.1 [2]
vs. chilled	5	8.2	1.6 [1]	....	....	....
vs. sharp-frozen	5	7.6	2.2 [2]	25	7.0	2.2 [2]
Brine-spray *vs.* chilled	....	....	0.4	....	....	....
Brine-spray *vs.* sharp-frozen	....	....	1.0	....	....	1.1 [2]
Chilled *vs.* sharp-frozen	....	....	0.6	....	....	....
2. *Liver aroma*						
Fresh	5	9.4	....	25	9.0	....
vs. brine-spray	5	8.0	1.4 [1]	25	7.8	1.2 [2]
vs. chilled	5	7.2	2.2 [2]	....	....	....
vs. sharp-frozen	5	5.6	3.8 [2]	25	6.4	2.6 [2]
Brine-spray *vs.* chilled	....	....	0.8	....	....	....
Brine-spray *vs.* sharp-frozen	....	....	2.4 [2]	....	....	1.4 [2]
Chilled *vs.* sharp-frozen	....	....	1.6 [2]	....	....	....

[1] Significant. [2] Highly significant.

In thigh, breast, and giblet flavor the order of scores followed the same pattern as before—fresh, brine-spray, chilled, sharp-frozen. The differences here were generally significant in both the series involving all treatments and the series involving freezing methods, except between the chilled and brine-spray treatments. The quality of the birds in the chilled and brine-spray series was practically identical.

The unusually low scores for the giblets from all treatments except the fresh controls were unexpected and merit extensive study to learn the nature of the changes involved and to determine the factors which control these changes. Further work on this point is under way. Study indicates that the quality of the frozen liver deteriorates rapidly. In this study this fact accounted for the very low score of the gizzards since they were cooked in the same water and at the same time.

TABLE 2B

Palatability Scores of Poultry Handled in Various Ways
(Six Months' Storage)

Treatment	Number of birds	Breast flavor		Breast juiciness		Thigh flavor		Thigh juiciness		Liver flavor		Gizzard flavor	
		Average score	Difference	Average score	Difference	Average score	Difference	Average score	Difference	Average score	Difference	Average score	Difference
All methods													
Fresh	5	8.68	......	7.18	......	8.32	......	8.20	......	7.54	......	8.06	......
vs. brine-spray	5	8.48	0.20	5.86	1.32 [1]	8.18	0.14	7.08	1.12 [1]	6.06	1.48 [1]	6.12	1.94 [2]
vs. chilled	5	7.76	0.92 [2]	6.14	1.04	8.08	0.24	6.88	1.32 [2]	6.00	1.54 [1]	5.66	2.40 [2]
vs. sharp-frozen	5	7.58	1.10 [2]	6.22	0.96	7.40	0.92 [2]	7.06	1.14 [1]	4.14	3.40 [2]	5.02	3.04 [2]
Brine-spray *vs.* chilled	5	......	0.72 [1]	......	0.28	......	0.10	......	0.20	......	0.06	......	0.46
Brine-spray *vs.* sharp-frozen	5	......	0.90 [2]	......	0.34	......	0.78 [1]	......	0.02	......	1.92 [1]	......	1.10 [1]
Chilled *vs.* sharp-frozen	5	......	0.16	......	0.08	......	0.68 [1]	......	0.18	......	1.86 [1]	......	0.64
Freezing methods													
Fresh	25	8.74	......	7.40	......	8.60	......	8.16	......	8.28	......	8.10	......
vs. brine-spray	25	8.16	0.58 [2]	5.77	1.63 [2]	7.72	0.88 [2]	6.84	1.32 [2]	5.28	3.00 [2]	6.20	1.90 [2]
vs. sharp-frozen	25	8.01	0.73 [2]	5.68	1.72 [2]	7.34	1.26 [2]	7.10	1.06 [2]	5.03	3.25 [2]	5.42	2.68 [2]
Brine-spray *vs.* sharp-frozen	25	......	0.15	......	0.09	......	0.38 [1]	......	0.26	......	0.25	......	0.78 [2]

[1] Significant. [2] Highly significant.

TABLE 3A

Aroma Scores During Preparation for Poultry Handled in Different Ways

(Frozen Birds Held One Year)

Treatment	Number of birds	Kidney area		Abdominal cavity	
		Average score	Difference	Average score	Difference
Fresh control	5	9.6	....	9.6	....
vs. brine-spray	5	7.4	2.2 [2]	7.2	2.4 [2]
vs. chilled	5	7.0	2.6 [2]	6.8	2.8 [2]
vs. sharp-frozen	5	6.2	3.4 [2]	5.9	3.7 [2]
Brine-spray *vs.* chilled	....	....	0.4	....	0.4
Brine-spray *vs.* sharp-frozen	....	....	1.2 [2]	....	1.3 [2]
Chilled *vs.* sharp-frozen	....	....	0.8 [1]	....	0.9
		Liver aroma		Aroma during-cooking	
Fresh control	5	8.8	....	10.0	....
vs. brine-spray	5	6.0	2.8 [2]	7.4	2.6 [2]
vs. chilled	5	5.2	3.6 [2]	6.8	3.2 [2]
vs. sharp-frozen	5	4.8	4.0 [2]	6.0	4.0 [2]
Brine-spray *vs.* chilled	....	....	0.8 [1]	....	0.6
Brine-spray *vs.* sharp-frozen	....	....	1.2 [2]	....	1.4
Chilled *vs.* sharp-frozen	....	....	0.4	....	0.8

[1] Significant. [2] Highly significant.

TABLE 3B

Palatability Scores on Poultry Handled in Different Ways

(Frozen Birds Stored One Year)

Treatment	Number of birds	Thigh flavor		Thigh juiciness	
		Average score	Difference	Average score	Difference
Fresh control	5	8.7	....	7.6	....
vs. brine-spray	5	7.7	1.0 [1]	6.4	1.3 [2]
vs. chilled	5	7.4	1.3 [2]	5.8	1.8 [2]
vs. sharp-frozen	5	6.0	2.7 [2]	6.1	1.5 [2]
Brine-spray *vs.* chilled	....	....	0.3	....	0.6
Brine-spray *vs.* sharp-frozen	....	....	1.7 [2]	....	0.3
Chilled *vs.* sharp-frozen	....	....	1.4 [2]	....	0.3
		Breast flavor		Breast juiciness	
Fresh control	5	9.1	....	7.9	....
vs. brine-spray	5	7.9	1.2 [2]	5.6	2.3 [2]
vs. chilled	5	7.7	1.4 [2]	6.3	1.6 [1]
vs. sharp-frozen	5	6.9	2.2 [2]	5.5	2.4 [2]
Brine-spray *vs.* chilled	....	....	0.2	....	0.7
Brine-spray *vs.* sharp-frozen	....	....	1.0 [1]	....	0.1
Chilled *vs.* sharp-frozen	....	....	0.8 [1]	....	0.8
		Liver flavor		Gizzard flavor	
Fresh control	5	8.5	....	8.8	....
vs. brine-spray	5	5.1	3.4 [2]	5.3	3.5 [2]
vs. chilled	5	4.9	3.6 [2]	5.5	3.3 [2]
vs. sharp-frozen	5	4.0	4.5 [2]	4.9	3.9 [2]
Brine-spray *vs.* chilled	....	....	0.2	....	0.2
Brine-spray *vs.* sharp-frozen	....	....	1.1	....	0.4
Chilled *vs.* sharp-frozen	....	....	0.9	....	0.6

[1] Significant. [2] Highly significant.

The juiciness scores for all the treatments involving freezing were lower than for the fresh controls. However, there was no significant difference between the two freezing methods (brine-spray or air) or between the birds frozen once (chilled series) and those frozen twice (brine-spray and sharp-frozen series). This dryness in texture of cooked chicken flesh which had been frozen merits further study. Work on this defect is now under way.

A microscopic study of the muscle fibers showed that some change occurred in the physical structure of stored frozen muscle. The elastic fibers of the connective tissues were most affected. They seemed to fall apart into rod-shaped fibers. This is shown in a photomicrograph by Hoffman (Fig. 1) and reported by Lowe (1939). This type of change occurring during frozen storage has been encountered only in *frozen* poultry. The most perceptible change in unfrozen broilers with aging occurred in the muscle fibers and not in the elastic tissues, according to Stewart, Lowe, and Morr (1941).

FIG. 1.

Twelve Months' Storage Period: The results of this series are, in general, similar to the results of the six months' studies. There was a further decline in score for all the stored birds compared with the fresh controls. The order of scores still remained fresh, brine-spray, chilled, sharp-frozen. Again the differences were not always significant.

The loss of flavor and the development of off-flavors in the giblets followed the same pattern as at six months' storage. However, the differences between treatments (except fresh controls) were not significant. The livers of all the frozen birds were very unpalatable.

The loss of juiciness in the frozen birds also followed the trend noted at six months' storage. The cooked muscle from all frozen birds became quite dry and had a sawdust-like feel in the mouth.

SUMMARY

Taking all of the results into consideration, it may be said that birds which are brine-spray frozen within five hours after dressing and stored up to six months before eviscerating (and handled in other ways according to the schedule given here) are superior in quality to birds eviscerated 18 hours after dressing and stored for the same period of time. An additional six months' storage of the eviscerated birds yielded similar results though the quality of all birds declined during the additional storage period. Sharp-frozen birds which were chilled for 18 hours and held up to six months before eviscerating were distinctly inferior to those in either of the above two treatments.

These results seem to indicate that any treatment which minimizes the length of time that undrawn poultry is held above freezing will aid in preventing off-odors and flavors in the carcass. This is in accordance with previous work by Stewart, Lowe, and Morr (1941), Sair and Cook (1938), and Fitzgerald and Nickerson (1939).

Further, off-odors and flavors and loss of juiciness, which were apparently independent of the treatments studied in these experiments, developed in all stored poultry. The changes in flavor and aroma were particularly noticeable in the flavor of the liver and in the juiciness of all edible portions.

Loss of juiciness in poultry meat which had been frozen was not influenced by freezing twice or by the differences in freezing rates used.

Present practice in eviscerating plants is to eviscerate chickens which have been dressed, chilled, and held from six to 96 hours (time required for shipment to eviscerating plant and for co-ordination of operations). By using the method described under brine-spray (Z process) series, it would be possible to store New York dressed poultry for eviscerating later with no loss of quality over present methods. This procedure would allow a plant to eviserate poultry the year around and not be limited to the usual four or five months of heavy marketing of live poultry.

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STUDY OF VARIOUS FACTORS AFFECTING PERMEABILITY OF BIRDS' EGGSHELL

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The shell of birds' eggs, besides giving physical protection to its contents, possesses several other distinctive functional properties. It controls the evaporation of moisture and preserves the natural colloidal state of albumen. The porous condition of the shell is to a great extent responsible for the rapid deterioration of eggs. During incubation this condition also regulates the rate of (1) the gaseous exchange, (2) the admission of external heat when the egg is warmed and emission of heat when it is cooled, and (3) the dissipation of metabolic heat. The experimental study of the porous condition, or specifically the permeability, of the eggshell, therefore, is of biological and economic interest. Such a study may suggest new efficient methods of preservation of eggs both for hatching purposes and for human consumption, as well as reveal some causes leading to the death of the embryo.

Perhaps among the first to observe that the eggshell has pores were Baudrimont and Martin-St.-Auge (1847). Soon after, Wittish (1851) and Nathusius (1868) made a detailed morphological study of pores, and later Rizzo (1899) carefully counted them. The nature, size, and distribution of pores under various conditions were further elaborated in studies of Romanoff (1929), Almquist and Holst (1931), and others.

The direct measurement of permeability to water was attempted by Thumberg (1902); to various gases, using small pieces of shell, by Hüfner (1892); and to air, using somewhat larger pieces, by Camus (1904), Ferdinandoff (1931), and Penionschkevitch (1937). The water method on the whole was found to be very slow and inaccurate, Romanoff (unpublished). The passage of air or of pure gases through a relatively small piece of shell, without adequate control of its state of dryness, also did not give good, reproducible results. Therefore, there were distinct needs for a better method for the direct measurement of permeability and a more detailed knowledge about the variation in permeability caused by hereditary and environmental factors.

EXPERIMENTAL PROCEDURE

For this investigation 1,307 eggs, laid by a flock of White Leghorn hens *(Gallus domesticus)*, were used. The average weight of these eggs was 60.5 ± 2 grams. To avoid possible influence of aging on shell permeability all eggs were usually used the next day after being laid. In addition to chicken eggs a few eggs of other species of birds (geese, turkeys, ducks, guinea fowls, pheasants, grouse, and quails) were used.

The egg to be studied was broken into halves, emptied of its contents, washed thoroughly in a forced stream of lukewarm water (on the inside only to prevent the removal of cuticle), and finally dried.

The permeability of eggshell to air or to pure gases (oxygen, nitrogen, carbon dioxide, and hydrogen) was measured directly by means of a perfected apparatus (Fig. 1). This apparatus produces the constant suction of a gas from the inside of an end portion (about six square centimeters)[1] of the eggshell (B). The passing gas (A) was measured in a specially designed micro-gas meter (C) by the displacement of water. The constant partial vacuum was maintained automatically, irrespective of the rate of gas flow through the shell, by an electrically operated manostat (F).

FIG 2. FIG 1.

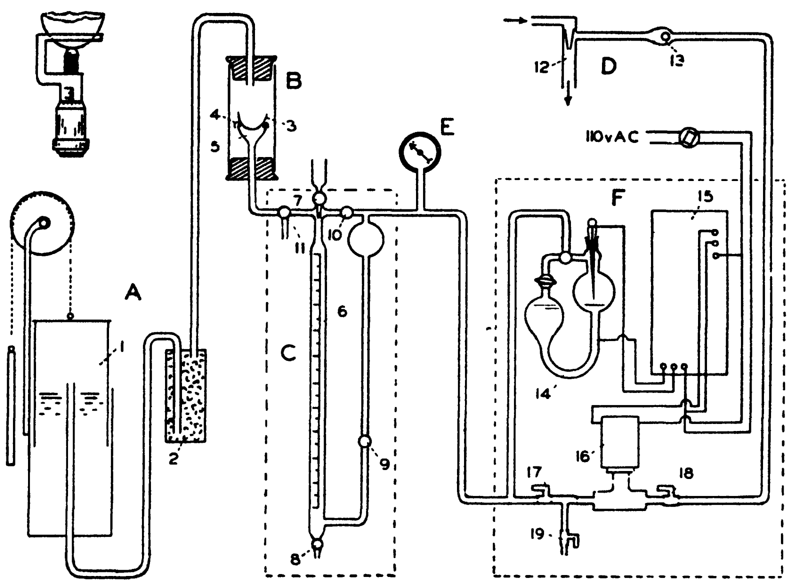

FIG. 1. A diagram of the apparatus for measurement of the permeability of a gas through birds' eggshell. A—gas holder (1) with a gas, leading to a drying tube (2); B—eggshell chamber, shown with eggshell (3), rubber gasket (4), and funnel (5) of one-inch inside diameter; C—micro-gas meter, consisting of a 50-c.c. burette (6), funnel with glass stopper (7) for refilling of the burette (6) with water, drain stopcock (8), control stopcock (9), set up stopcock (10), and three-way vacuum relief stopcock (11); D—vacuum pump, including water suction pump (12) and back pressure valve (13); E—vacuum gauge; F—automatic vacuum controlling mechanism, consisting of sulphuric acid monostat (14) capable of maintaining a vacuum constant to ± .015 mm. of mercury, electronic relay (15) for 120 volts A.C., solenoid valve (16), with control (17 and 18) and bleeder (19) valves.

FIG. 2. A micrometric-spherometer shown with a portion of eggshell, the surface of which is to be measured.

It was found, however, that a fairly constant partial vacuum of the system could also be obtained by manual control with the use of the base of a Bunsen burner, or still better of a gas v-point needle valve connected in place of the automatic control mechanism.

[1] In a test experiment it was found that the air flow in the shell is much greater from inside than from outside. This seems to be in agreement with the Lucke, Hartline, and McCutheon (1931) experiments on the permeability of *Arbacia* eggs to water, that is, that permeability is greater during exosmosis than during endosmosis.

The area of the observed portion of the eggshell was calculated using the formula for the area of the curved surface of a spherical segment:

$$\text{Area} = 2\pi \text{Rh}$$

where $R = \dfrac{h^2 + r^2}{2h}$, h is the height of the curved surface of the shell, which was measured by a special micrometer-spherometer (Fig. 2), and r is the radius of the receptacle (5) (Fig. 1) which was equal to one-half inch (about 1.25 cm.). The flow of a gas through a known surface area in a given unit of time can then be easily converted into comparable values which may read in cubic centimeters of gas/cm.^{2} of shell/minutes of time.

In the course of preliminary experiments it was found that drying of

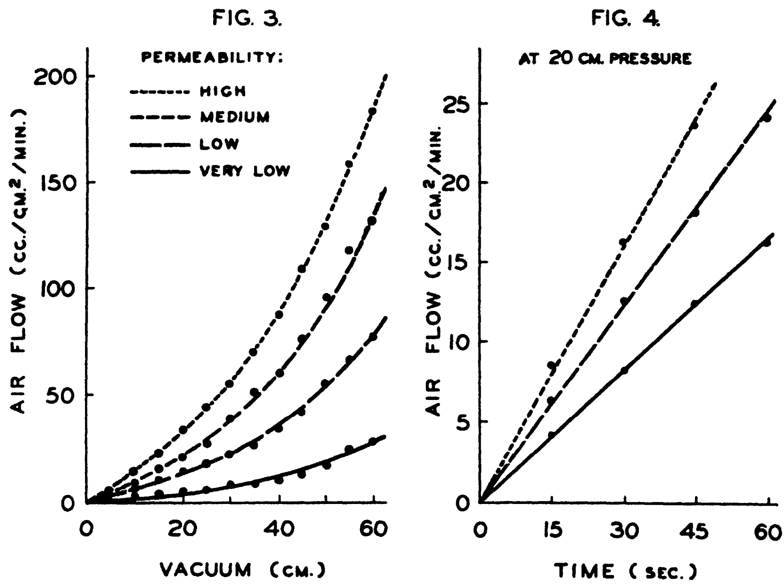

FIG. 3. Changes in the rate of air flow with the increase in vacuum.
FIG. 4. Changes in the rate of air flow with time.

shells is necessary in order to reach their more or less stable condition and to obtain comparable results. It was observed that the permeability of the wet shell was only about eight per cent of that of the well-dried one. After 24 hours of drying at a room temperature of 20°C.(68°F.) the shell permeability was still about 50 per cent; at 55°C.(131°F.) about 80 per cent; at 55°C. with a vacuum, over 90 per cent; and at 100°C.(212°F.) the permeability reached almost 100 per cent of that of the dried shell. In the final experiments the drying was carried out for 48 hours at 55°C. with a 62.5-cm. vacuum.

It was also observed that the rate of air flow through the eggshell increases with the increase of partial vacuum (Fig. 3). This increase was not lineal but gave an exponential curve. The maximum vacuum that could be applied to the weakest shell without rupturing it was found to be 20 centimeters. Therefore, this vacuum was used throughout the final experiments.

The continuous measurements of the rate of air flow with time gave a linear increase in values (Fig. 4). This would indicate that the apparatus employed produced comparable results irrespective of the actual permeability of the eggshell.

EXPERIMENTAL RESULTS

Role of Inner Shell Membrane: Hüfner (1892) did not find any effect of the removal of the inner shell membrane on the rate of passage of air through the eggshell. In the present experiment with hens' eggs it was observed that the removal of this membrane always resulted in an increase of shell permeability (Table 1). The increase was on an average about nine per cent, and it varied in different eggs from three to 17 per cent. The relatively small difference in permeability with and without the membrane would suggest that the principal barrier of the shell is its amorphous calcareous layer.

TABLE 1

Influence of Removal of Inner Membrane on Flow of Air Through Eggshell

Observations	With inner membrane	Without inner membrane	Difference	
	c c /cm ²/min	c c /cm ²/min	c c /cm ²/min	pct
1	21.88	24.63	2.75	12.57
2	10.78	12.62	1.84	17.07
3	16.88	17.82	0.94	5.57
4	15.60	16.05	0.45	2.88
5	23.23	25.05	1.82	7.83
Average..................	17.67	19.23	1.56	8.83

Owing to technical difficulties in removing the membranes from the shell, especially at the sharp end of the egg, all observations in this study of shell permeability were made with both the inner and outer membranes.

Retention of Water by Pores in Relation to Their Volume and Area: In determining the relation between permeability of eggshell to air and the amount of retention of water in the pores the following procedure was adopted. The half eggshell of known permeability was dried and weighed on an analytical balance. Then it was filled with distilled water under suction in the apparatus (Fig. 1) for one minute. The shell was then emptied of water, dried with a clean dry cloth to remove moisture, and quickly weighed. The difference between this and the former weight gave the amount of water held by the pores. These values in general agreed with the observed rate of permeability (Table 2).

The volume of pores per cm.^{2} can be calculated from the specific gravity of water at room temperature. This would give 8.34×10^{-3}, 8.40×10^{-3}, and 9.68×10^{-3} pore volume per cm.^{3} in low, medium, and high permeability shells, respectively. Now, assuming that these pores are of uniform diameter, their area can be obtained from the volume of pores divided by the thickness of eggshell (.311 mm.)—Romanoff (1929). The pore area of these three kinds of shells would be 2.38×10^{-3}, 2.40×10^{-3}, and 2.77×10^{-3} cm.^{2} If the total area of the shell is known the per cent of the total area which is open can also be calculated.

Difference in Permeability Between Blunt and Sharp Ends: The shell permeability was found to be different at each end of the egg. From the 191 eggs examined the average permeability of the blunt end was 14.52 c.c./cm.^{2}/min., while that of the sharp end was 10.46 c.c./cm.^{2}/min. The difference in permeability of the two ends of the egg was nearly 28 per cent. Further study of the data (Table 3) indicates that there is a considerable variation in shell permeability of fowls' eggs. Two groups of eggs with very low permeability showed that the sharp end was more permeable than the blunt end. The difference in permeability of the first

TABLE 2

Relation Between Permeability and Water Retention of Eggshell

Groups of eggs	Number of eggs	Permeability of eggshell	Water retention
		c.c./cm.^{2}/min.	*gm.*
Low permeability	18	9.24	.0618
Medium permeability	22	16.73	.0629
High permeability	20	26.46	.0717

group of eggs was as high as 52 per cent. In all other eggs of higher permeability the sharp end was less permeable than the blunt one. The difference usually increased with the increase of permeability. In a small group of extremely permeable eggs this difference reached 51 per cent in favor of the blunt end.

TABLE 3

Difference in Air Permeability Between Blunt and Sharp Ends of Hens' Eggs

Permeability of blunt end (class intervals)	Number of eggs (frequency)	Permeability of eggshell		
		Blunt end	Sharp end	Difference
		c.c./cm.^{2}/min.	*c.c./cm.^{2}/min.*	*pct.*
0.0- 5.0	13	3.46	7.21	52.01
5.1-10.0	46	7.68	8.11	5.30
10.1-15.0	45	12.27	8.39	—31.62
15.1-20.0	43	17.40	12.29	—29.37
20.1-25.0	28	22.21	12.95	—41.69
25.1-30.0	13	27.48	16.52	—39.88
30.1-35.0	3	32.07	15.70	—51.04
Total	191	......	......	......
Average	......	14.52	1.46	—27.96

Intra- and Interindividual Variation: The permeability among the eggs of individual birds varied from .9 to 4.1 c.c./cm.^{2}/min. The average intraindividual variation for the blunt end was ± 2 and for the sharp end ± 1.4 c.c./cm.^{2}/min. This indicates that the permeability of the blunt end was somewhat more variable than that of the sharp end.

From the study of interindividual variation of 16 birds with four to 20 eggs for each bird, it was found that the average permeability for different hens varied from 7.7 to 29.2 c.c./cm.^{2}/min. for the blunt end, and from 5.3 to 14.3 c.c./cm.^{2}/min. for the sharp end of the egg. The average permeability for all birds was 15 ± 1 and $8.3 \pm .7$ c.c./cm.^{2}/min. for the sharp and blunt ends, respectively.

Changes With Age of a Hen: It is of interest to know whether any changes in the permeability of eggshell occur with the age of the hen. In this investigation an average of three eggs per hen from 203 hens of various ages were tested for permeability. Slight decreases are indicated (Table 4) in the rate of air flow as the pullet becomes a hen one year and two years old. The decreases on the blunt and sharp ends are at approximately the same rate. These changes are too negligible, however, to be of great significance.

Relation to Thickness and Breaking Strength: An analysis of the data of 236 examined fresh eggs showed no relation between the rate of air

TABLE 4

Changes in Shell Permeability With Age of Hens

Age of hen	Number of hens	Permeability of eggshell	
		Blunt end	Sharp end
		c.c./cm.²/min.	*c.c./cm.²/min.*
Pullet	153	13.34	10.76
One year old	97	13.08	9.99
Two years old	13	12.64	8.81

flow through the shell and the shell thickness. Since the thickness and the breaking strength of the eggshell are directly correlated with each other—Romanoff (1929), Stewart (1936), and Lung, Heiman, and Wilhelm (1938)—it is possible to assume that the rate of air flow through the shell is entirely independent of these other two factors. From this it is very evident that permeability is an independent physical property of eggshell.

TABLE 5

Influence of Position of Hen's Egg in Laying Cycle on Permeability of Its Eggshell to Air

Length of laying cycles	Number of cycles	Permeability of eggshell (c.c./cm.²/min.)							
		Blunt end				Sharp end			
		1st egg	2nd egg	3rd egg	Change	1st egg	2nd egg	3rd egg	Change
					pct.				*pct.*
Two-egg cycle	28	14.70	18.22	......	19.2	12.62	10.38	......	—17.7
Three-egg cycle	6	14.46	15.06	17.06	15.2	19.54	8.26	6.92	—64.6

Position of Egg in Laying Cycle: The data show that the permeability of eggshell is subject to change with the successive eggs of a laying cycle (Table 5). Both in the two-egg and in the three-egg cycle the changes of blunt and sharp ends of the egg were in the opposite direction. The permeability of the blunt end increased, while that of the sharp end decreased with the successive eggs of the cycle. The total increase in permeability of the blunt end in the two-egg cycle was 19.2 per cent and in the three-egg cycle, 15.2 per cent. The total decrease in permeability of the sharp end was 17.7 per cent in the two-egg cycle and as high as 64.6 per cent in the three-egg cycle.

Changes With Incubation: The examination of 90 fresh and 77 fertilized incubated eggs from the same eight hens indicates that the shell per-

meability increases with incubation (Table 6). The increase was especially pronounced in the blunt end of the egg. During 18 days of incubation the average increase in permeability of the blunt end was 26.91 per cent, while that of the sharp end was three times less, or on an average, only 8.6 per cent. This would indicate that the permeability of the eggshell increases with progressive stages of embryonic development more rapidly at the blunt—the usual seat of air cell—than at the sharp end.

TABLE 6

Influence of 18 Days of Embryonic Development of the Chick on Change in Permeability of Eggshell [1]

Condition	Number of eggs	Permeability of eggshell					
		Blunt end	Sharp end	Difference		Per cent difference	
				Blunt ends	Sharp ends	Blunt ends	Sharp ends
		c.c./cm.²/min.	*c.c./cm.²/min.*				
Fresh eggs	90	15.43	13.18	5.68	1.24	26.91	8.60
Developed 18 days	77	21.11	14.42				

[1] Both fresh and developed eggs were obtained from the same group of eight hens.

Aging and Environmental Conditions: Ordinarily the shell permeability changes with aging. In order to determine the direction and the rate of changes under different environmental conditions the groups of dried shells were exposed for one week to various combinations of high and low temperatures, humidity, and level of carbon dioxide. The data so obtained

TABLE 7

Influence of One Week of Exposure to High and Low Temperature, Humidity, and Concentration of Carbon Dioxide on Changes in Permeability of Hen's Eggshell [1]

Temperature	Humidity	Carbon dioxide	Permeability of eggshell to air			
			Before exposure	After exposure	Changes in permeability	
			c.c./cm.²/min.	*c.c./cm.²/min.*	*c.c./cm.²/min.*	*pct.*
37.5°C.(99.5°F.)	High	High	17.10	35.28	18.18	106.32
37.5°C.(99.5°F.)	High	Low	15.37	25.38	10.01	65.13
37.5°C.(99.5°F.)	Low	Low	12.50	16.35	3.85	30.80
37.5°C.(99.5°F.)	Low	High	15.97	15.02	—0.97	—6.07
20.5°C.(69°F.)	High	High	15.33	24.21	8.88	57.93
20.5°C.(69°F.)	High	Low	16.61	21.96	5.35	32.21
20.5°C.(69°F.)	Low	Low	15.89	16.94	1.05	6.61
20.5°C.(69°F.)	Low	High	18.21	17.73	—0.48	—2.64

[1] There were 10 eggs in each lot.

(Table 7) reveal that under certain cónditions the permeability of the eggshell considerably increases, while under others it slightly decreases. Singularly, high temperature and high humidity invariably increased shell permeability. With a combination of several factors the greatest increase, over 106 per cent, was observed under high temperature, high humidity, and high concentration of carbon dioxide. A slight decrease in permeability, from 2.6 to 6 per cent, was found in the combination of either high

or low temperature with low humidity and high concentration of carbon dioxide. It may be noted that the high carbon dioxide concentration in combination with low humidity played an important role in the changes of permeability of eggshell.

Relative Permeability to Various Gases: Hüfner (1892) observed that hydrogen penetrated eggshell most easily, then carbon dioxide, then nitrogen, and lastly oxygen.

The data of the present study (Table 8) fully confirm Hüfner's observations. Oxygen was found to pass through fresh shell over seven per cent more slowly than air. Nitrogen passed slightly faster than air. Carbon

TABLE 8

Relative Permeability of Hen's Eggshells From Fresh and Developed Eggs[1] to Air, Nitrogen, Oxygen, Carbon Dioxide, and Hydrogen

Gases	Actual permeability		Relative permeability	
	Fresh eggs	Developed 18 days	Fresh eggs (air 100)	Developed 18 days (air 100)
	c.c./cm.^{2}/min.	c.c./cm.^{2}/min.	c.c./cm.^{2}/min.	c.c./cm.^{2}/min.
Air	16.73	23.49	100.00	100.00
Nitrogen (N_2)	16.87	23.76	100.86	101.15
Air	16.73	23.48	100.00	100.00
Oxygen (O_2)	15.53	22.43	92.85	95.49
Air	16.71	23.51	100.00	100.00
Carbon dioxide (CO_2)	17.26	24.51	103.19	104.34
Air	16.72	23.46	100.00	100.00
Hydrogen (H_2)	23.58	35.89	140.98	152.79
Air	16.74	23.49	100.00	100.00
Air (average)	16.73	23.49	100.00	100.00

[1] Based on observations of 18 fresh and 12 developed eggs from the same hen. The successively laid eggs were used alternatively for testing as fresh or as incubated (developed).

dioxide passed over three per cent faster than air and over 10 per cent faster than oxygen. Hydrogen passed over 40 per cent faster than air.

The permeability of eggshell to pure gases increased with incubation as was the case with air. The rates of the flow of air and gases into incubated eggs were nearly in the same proportions as to fresh eggs.

The increased permeability of eggshell with incubation is of great biological significance to the developing embryo. It facilitates the exchange of respiratory gases especially when the metabolism increases with the growth of the embryo. Also the more rapid escape of carbon dioxide than the entrance of oxygen may be interpreted as the biological protection against asphyxia which can occur at a relatively high pressure of carbon dioxide, according to the work of Romanoff (1930) and Romanoff and Romanoff (1933).

Permeability Among Eggs of Different Species: It is of interest to compare the permeability of eggshells of different species. Hüfner (1892) indicated that hen's eggshell is less penetrable than goose's eggshell. Our data on eggshell permeability of goose, turkey, duck, fowl, guinea fowl, pheasant, grouse, and quail are in descending order from 35 with goose egg to 3 c.c./cm.^{2}/min. with quail egg (Table 9).

The fact is that the permeability values in general are high in large eggs and that they fall off with the decrease in size of the eggs. Presumably there is a biological necessity for the large egg to be more permeable since its surface area is smaller in proportion to its weight. This fact is especially important for the maintenance of the proper rate of evaporation and of the equilibrium between the water and dry substance of the egg in the course of embryonic development.

TABLE 9

Permeability of Eggshell of Various Species of Birds

Species of birds	Permeability to air
	c.c./cm.²/min.
Embden goose *(Anser domesticus)*	35.0
Bourbon red turkey *(Meleagris gallopavo)*	21.0
Runner duck *(Anas domesticus)*	20.0
Leghorn fowl *(Gallus domesticus)*	19.5
Guinea fowl *(Numida meleagris)*	19.0
Ringnecked pheasant *(Phasianus torquatus)*	10.0
Ruffed grouse *(Bonasa umbellus)*	5.0
Bobwhite quail *(Colinus virginianus)*	3.0

DISCUSSION

The present method of measuring shell permeability is highly accurate and, though it took nearly seven years to perfect, it is comparatively simple. The observations which may be made give comparative values that are suitable for quantitative analysis. Furthermore, they are readily reproducible under a given set of laboratory conditions.

The results of shell permeability so obtained are unfortunately only relative, but if it were possible to measure the actual permeability of the eggshell *in situ,* it is questionable whether such measurements would be accurate enough to be of any scientific interest. Thus the values to be observed on a necessarily limited surface area of the shell would be relatively small and extremely variable because of the instability in both atmospheric and internal vapor pressure. In addition several other physical as well as biological factors, dealt with in detail in the present study, would greatly interfere with the accuracy of the results. Indeed, the accurate quantitative data as obtained from the dissected eggs by the described method may be of much greater value in the studies of various aspects of variation in shell permeability.

The present studies lead to the apprehension of the fact that the eggshell is not uniformly permeable over its entire surface as observed from the differences between the blunt and the sharp ends (Table 3). Furthermore, in highly porous shells the permeability is much greater at the blunt than at the sharp end, while in less permeable shells this condition may be reversed, or at least the ends may be more nearly identical.

Permeability, like many other physical properties of the eggshell, is consistent with the hen. In the light of this fact the average permeability of eggshells may be easily determined for individual hens. Also, in this way, it is possible, on the basis of only a few tested eggs to select hens which produce eggs with the desired quality of shell. Since the permea-

bility of the shell is not correlated with either the breaking strength or the thickness, the problem of selection for this shell quality would require the development of a new apparatus capable of measuring shell permeability in fresh, unbroken eggs.

Another factor influencing permeability is the cyclical laying of the eggs. The values between any two egg cycles are much greater than those between any successive laying. Consequently each cycle of laying is preceded by a brief physiological rest. The effect of this rest is immediately reflected in the formation of a larger, more morphologically complete egg, owing to a more actively functioning ovary and, especially, oviduct. It has been shown by Atwood (1929) and Bennion and Warren (1933) that the first egg of the cycle is heaviest, and the present data (Table 5) show that it has pores uniformly distributed.

In the study of eggshell permeability it is necessary to deal with the entire shell structure, including not only the calcareous portion but also the structureless gelatinous cuticle on the surface and in the pores, and the thin double shell membrane on the inside. All of these distinct layers were considered as one part of the shell in the present study. Such an approach was found to be necessary because Penionschkevitch (1937) pointed out that the permeability of the shell membranes is of considerable importance in the practice of incubation, particularly in the control of temperature, humidity, and ventilation, and in the study of causes leading to embryonic mortality. He found that during incubation the moisture content of the shell membranes increases, while their permeability to gas decreases. Drying out of the membranes results in an increase in their permeability, contrary to previous data of Ferdinandoff (1931).

The function of the cuticle in shell permeability, as was emphasized by Marshall and Cruickshank (1938), is to accelerate the evaporation, not to retard it. It is also important in relation to the interchange of respiratory gases.

The fact that there is more rapid passage of a heavy gas, carbon dioxide, than of a light gas, oxygen (Table 8), is of extraordinary interest from the biological point of view. It would seem that the eggshell balances the passage of respiratory gases to meet the physiological requirements of the embryo, contrary to the physical law of the rates of gaseous diffusion. Hüfner (1892) suggested that the penetration of gases through the eggshell follows Graham's law, that is, it is inversely proportional to the square roots of their densities, but his data failed to demonstrate this. However, Ancel (1928, 1929) showed that the penetration of chloroform vapor did follow this law.

The present data on permeability of the shell to gases under pressure (Table 8) demonstrate the adherence to Graham's law with the exception of the rate of passage of carbon dioxide which, although more dense than oxygen, passes more rapidly through the eggshell. This phenomenon may be related to an acid-base change of the gelatinous substance in the pores of the shell since it is known that loss of carbon dioxide from the egg causes a rise in alkalinity of the albumen, Romanoff (unpublished).

On the whole, from this study it is obvious that the shell permeability is not a fixed physical property. Ordinarily it increases with the age of the

egg and with incubation. However, under certain environmental conditions, such as low humidity and high carbon dioxide pressure (Table 7), it may become less permeable. This fact is of particular interest in the preservation of table eggs because the porous condition of the shell is largely responsible for the rapid deterioration of the egg contents. Any method reducing the evaporation of water through the shell at low humidities, when the invasion of molds is impossible, would be of immense economic importance.

SUMMARY

From the study of continuous flow of a gas through birds' eggshells with a perfected apparatus it was observed that the permeability of the shell varies as follows:

(1) It is lower in the presence of the inner membrane; (2) is more permeable at the blunt end than at the sharp end; (3) has direct relationship with the amount of retained water in the pores; (4) increases at the blunt end and decreases at the sharp end with successive eggs in the laying cycle; (5) is constant among the eggs of any one hen; (6) has no relationship to either breaking strength or thickness; (7) increases with embryonic development more rapidly at the blunt than at the sharp end; (8) is less permeable to oxygen and more permeable to nitrogen, carbon dioxide, and especially to hydrogen than to air; (9) the permeability increases with age at high humidity and carbon dioxide, and decreases at low humidity and high content of carbon dioxide; and (10) is higher in large eggs, like those of geese, and is lower in small eggs, like those of grouse or quail.

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STUDIES WITH BRANDY. I. pH [1]

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Although there is fair agreement as to the nature of the titratable acidity changes during the aging of distilled spirits in wooden containers, there is comparatively little information concerning the related pH fluctuations. Only recently have there been any detailed studies for whisky. Liebmann and Rosenblatt (1942) have found a knowledge of the pH useful in controlling the precipitation of metals from whisky. They reported that the change in pH during aging was a direct maturing characteristic of the whisky. They also showed that the pH tended to decrease during the first 24 months of storage. The pH of seven whiskies varied from 3.68 to 4.78, the highest value being in a new whisky. Valaer (1940) has studied the pH of commercial samples of Scotch whisky. In 97 samples of Scotch he found the pH to range from 4 to 4.78, averaging 4.34. In 70 American-Scotch type whiskies he found the pH to range from 4 to 6.22, averaging 4.81. In 10 samples of Irish whisky the pH ranged from 4.25 to 5.06, averaging 4.55. In rum Valaer (1937) found the pH to vary according to the source, exceeding 5 for Cuban and Puerto Rican rums. The pH of rums distilled in this country was lower. During aging there was generally a decrease in pH.

Valaer (1939) has also published the most extensive data on the pH of brandy. In 113 samples, primarily of post-prohibition California brandy, he found the pH to range from 3.85 to 5.67, averaging 4.42.[2] The low pH values of young brandies were attributed to the use of distilling material high in sulfur dioxide. A portion of the sulfur dioxide passing through the still is dissolved in the distillate and is slowly oxidized from sulfurous acid to sulfuric acid. Presumably this is not always the cause of low pH in brandies, for in 20 authentic French cognacs, in which the sulfate content owing to oxidation of sulfite is relatively low, the pH was found to vary from 3.76 to 4.98, averaging 4.14. In 25 Greek brandies the pH ranged from 3.28 to 5.77, averaging 4.41. Valaer also analyzed a number of apple, apricot, and peach brandies. In 85 apple brandies the pH averaged 4.67, in nine peach brandies 4.72, and in four apricot brandies 4.09. He also followed the pH change in seven grape brandies during aging. The pH dropped an average of 1.82 in four years in three brandies, but in four other brandies aged for two years the pH dropped only .60.

[1] This is the first of a projected series of articles on the composition of brandy. These studies were made under the direction of the senior author, but owing to the exigencies of war only this and a second paper on tannin can be published at the present time.

[2] pH's as low as 2.24 and as high as 7.97 were found in certain anomalous samples reported by Valaer in a private communication giving in detail the original data.

The original high pH of two of the samples aged for four years probably accounts for some of the difference.

It is therefore evident that the pH of commercial distilled spirits ranges from 4 to 5, that it tends to decrease during aging, and it appears that rum has a higher average pH than the other distilled spirits.

EXPERIMENTAL PROCEDURE

A Beckman pH meter was used for making the pH measurements and the titration curves. The instrument was regularly checked against standard buffer solutions. Schicktanz and Etienne (1937), in making whisky titration curves, indicated that dilution and other errors affect the accuracy of the pH measurements. Liebmann and Rosenblatt (1942), however, have found the glass electrode to give an accurate measure of the pH in 50 per cent alcoholic solutions up to a pH of about 8.

Brandies from three general sources were used. Ten samples were brandies submitted for judging at the 1939 Golden Gate International Exposition, hereafter called Exposition brandy. Unopened duplicate bottles were secured for analysis. Nineteen samples secured from the Growers Grape Products Association were produced in connection with the 1938 California grape prorate program.[3] These are called Prorate brandies hereafter and may be considered to give a representative picture of the composition of newly distilled, cut, caramelized California brandies. Eighty-four samples of brandy were produced in this laboratory during 1939 and 1940 in a 26-plate, 12-inch, Krenz-type, continuous column still. These brandies were produced from distilling material of known composition, were distilled under carefully observed and controlled operating conditions, were all cut to approximately 102° proof before barreling, and were aged at a constant temperature in cooperage treated in various ways. These brandies are reported as University brandies with their original cellar numbers.

EXPERIMENTAL RESULTS

The results of the analysis of the 10 Exposition brandies (Table 1) show a range from 3.38 to 4.47 in pH, averaging 4.10. Their total acidity is practically all volatile and ranges from .0193 to .0658, averaging .0404 per cent. There is no exact relationship between the titratable acidity and the pH except that with high titratable acidity the pH tends to be lower.

The pH and total acid determinations on the 19 Prorate brandies (Table 2) show a range in pH from 3.12 to 7.55, averaging 5.06. The total acidity averaged only .0083, and there was practically none in several of the samples, particularly in those with a pH exceeding 6. Some of

[3] The history of these samples is as follows: After the brandy produced in each run had been cut and colored, the storekeeper-gauger of the Alcohol Tax Unit took a sample from one of the barrels of the run. These samples were then sent to the association for organoleptic examination. A run usually consisted of 25 to 150 barrels. At the end of the season the unused portion of the samples from each of 19 distilleries, located in all parts of the state, were combined into 19 separate lots. Each lot represented an average of 15 different distillations made during the season, so that over 250 different distillations are represented in these composite samples.

these abnormally high pH's are probably due to the distillation of neutralized distilling material and the consequent lack of volatile acids in the distillate. The buffer capacity of new alcoholic distillates is so low that the addition of only small quantities of either acid or alkaline substances results in abnormally high or low initial pH; for example, use of alkaline water for cutting may result in high pH. Caramel syrups are not stable in alkaline solutions and the brandies with a high pH precipitated most of their caramel as a gummy, reddish mass. Newly distilled brandies with a pH below 4 are also abnormal. Valaer (1939) found a number of the young California brandies of very low pH and, as already mentioned, he

TABLE 1

Total Acidity and pH of the Exposition Brandies[1]

Number	Total acidity[2]	Volatile acidity[2]	pH
E-1	.0322	.0273	3.38
E-2	.0276	.0227	4.22
E-3	.0480	.0423	4.08
E-4	.0575	.0401	3.88
E-5	.0390	.0382	4.32
E-6	.0193	.0144	4.12
E-7	.0257	.0206	4.43
E-8	.0515	.0417	4.01
E-9	.0658	.0582	4.10
E-10	.0377	.0340	4.47
Average	.0404	.0340	4.10

[1] Proof ranged from 83° to 100°. [2] As grams of acetic acid per 100 ml.

TABLE 2

Acidity and pH of 19 Composite Samples of Young Brandies From the Prorate Brandies

Number	Number of samples in composite	pH	Total acidity[1]
P-1	14	5.08	.0036
P-2	14	3.28	.0216
P-3	15	7.50	.0004
P-4	12	7.43	.0010
P-5	19	3.88	.0098
P-6	15	3.60	.0272
P-7	15	6.45	.0009
P-8	?	4.40	.0058
P-9	14	6.30	.0013
P-10	16	3.12	.0314
P-11	14	4.85	.0017
P-12	15	4.11	.0055
P-13	16	7.55	.0005
P-14	15	4.28	.0068
P-15	11	4.72	.0023
P-16	15	6.17	.0020
P-17	14	3.52	.0114
P-18	13	3.32	.0234
P-19	16	6.65	.0004
Average	15	5.06	.0083

[1] As grams per 100 ml.

explained this on the basis of their high sulfurous-sulfuric acid content.

The analytical data for the 84 University brandies are summarized (Table 3). No sulfur dioxide was used in the fermentation of the distilling material for these brandies. Storage in five-gallon oak containers apparently speeded up the maturation as regards the volatile and total acids which became rather high. The pH is, however, not as low as that of commercial brandies which contain sulfurous-sulfuric acid and which are aged in 50-gallon barrels for approximately the same length of time.

During aging the pH of these brandies decreased, first rapidly, and after the first year more slowly. Originally the pH was approximately 5.

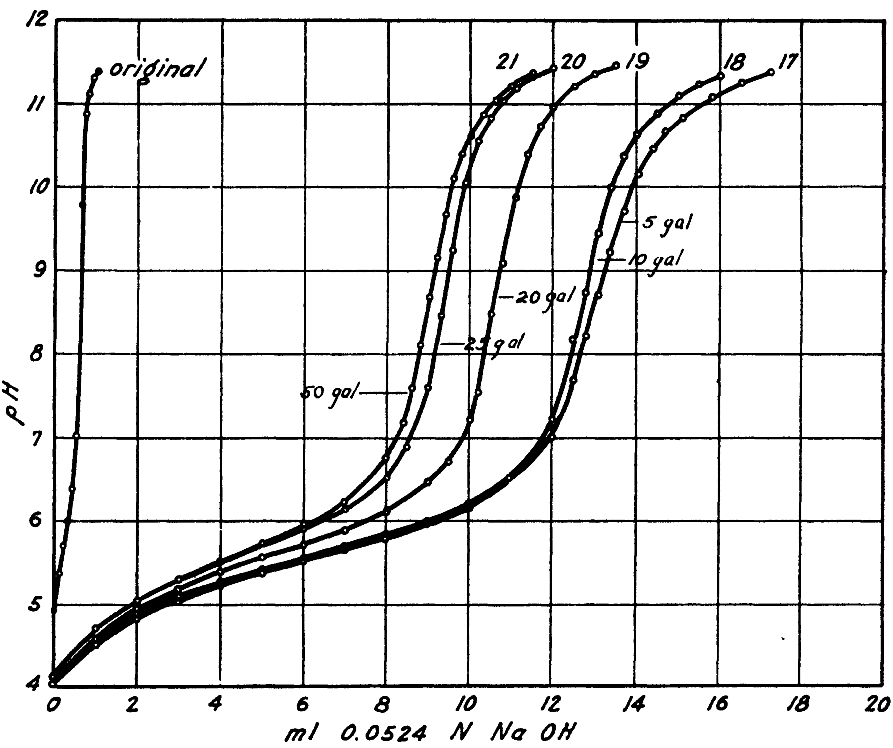

FIG. 1. Titration curves of the original brandy and of the same brandy after storage in five-, 10-, 15-, 25-, and 50-gallon containers.

During aging most of the brandies lost a full pH unit. In three special cases old wine, high in acetic acid, was distilled. The pH of the newly distilled brandy in these cases was 2.61, 3.78, and 3.91, and it increased during three years to 2.71, 4, and 3.98, respectively.

In the samples distilled from sound young wines the pH decreased and the shape of the titration curves changed during aging (Fig. 1). This change in shape of the titration curve during aging has been noted previously by Schicktanz and Blaisdell (1940) for whisky.

The data indicate that the larger the cooperage the less rapid the changes in pH and acidity. This is probably the result of at least three factors. The smaller barrels have a much greater surface exposed to the

TABLE 3

Influence of Age, Size, and Treatment of Barrels on Acidity and pH

University No.	Number of containers	Barrel size [1]	Barrel treatment [2]	Total acid (gm. acetic acid per 100 ml.) 1939 [3]	Total acid 1940	Total acid 1941	Total acid 1942	Volatile acid (gm. acetic acid per 100 ml.) 1940 [4]	Volatile acid 1941	Volatile acid 1942	pH 1939	pH 1940	pH 1941	pH 1942
1939 Brandies														
17	1	5	Rinsed with brandy	.0031	.0582	.0606	.0911	.0453	.0538	.0786	5.37	4.12	4.10	4.01
18	1	10	Rinsed with brandy	.0031	.0630	.0665	.0702	.0494	.0615	.0598	5.37	4.14	4.12	4.05
19	1	15	Rinsed with brandy	.0031	.0510	.0564	.0730	.0426	.0520	.0651	5.37	4.30	4.16	4.06
20	1	25	Rinsed with brandy	.0031	.0557	.0491	.0511	.0375	.0454	.0401	5.37	4.21	4.17	4.09
21	1	50	Rinsed with brandy	.0031	.0454	.0486	.0499	.0326	.0446	.0412	5.37	4.21	4.18	4.08
22	1	50	Charred; scraped out; rinsed with brandy	.0031	.0123	.0125	.0219	.0094	.0110	.0190	5.37	4.53	4.47	4.31
23	1	25	Two-year-old brandy barrel	.0031	.0162	.0168	.0304	.0129	.0147	.0243	5.37	4.72	4.70	4.45
27	1	5	Na_2CO_3 wash; soaked with brandy	.0031	.0529	0550	.0674	.0405	.0467	.0545	5.37	4.42	4.31	4.20
....	19	5	Rinsed with brandy	.0056	.0706	.0725	.0960	.0503	.0647	.0792	4.88	4.10	4.07	3.99
....	3	5	Steamed; soaked with brandy	.0034	.0618	.0665	.0859	.0436	.0595	.0678	5.40	4.11	4.10	4.00
1940 Brandies														
78	1	25	Steamed; soaked with brandy	....	.0031	.0342	.0466	....	.0312	.0415	......	4.98	4.37	4.30
79	1	25	Old brandy barrel	....	.0031	.0127	.0259	....	.0115	.0237	......	4.98	4.53	4.40
....	20	5	Boiled hot water; soaked with brandy	....	.0057	.0666	.0971	....	.0585	.0788	......	5.01	4.12	4.08
....	29	10	Boiled hot water; soaked with brandy	....	.0045	.0544	.0729	....	.0498	.0653	......	4.87	4.22	4.21

[1] In gallons. [2] All barrels were new unless stated otherwise. [3] Actual dates of withdrawal of sample, 1939 brandies; 1939 original brandy—Dec. 22 to Jan. 16, 1940; 1940—Aug. 15; 1941—Jan. 15; 1942—Jan. 19. 1940 brandies: 1940—Oct. 23 to Jan. 16, 1941; 1941—Aug. 4; 1942—Aug. 8. Note for the 1939 brandies that the time elapsed between the 1941 and 1942 analyses is one year, which is the same as that elapsing between the combined 1939, 1940, and 1941 analyses. [4] No volatile acid is given for the new brandy as it is approximately the total acid.

brandy per unit volume than the larger barrels. Therefore, more acidic compounds are extracted from the wood in the small containers. Moreover, the evaporation losses from small containers are more rapid so that greater amounts of the brandy are lost and larger air spaces develop in proportion to the total volume. Oxidation of alcohol to acetic acid is therefore accelerated. Finally, in the small containers, as the volume decreases more rapidly, there is a greater concentration of acidic materials. The final pH of the 1939 brandies stored in five-, 10-, 15-, 25-, and 50-gallon containers in 1942 was 4.01, 4.05, 4.06, 4.09, and 4.08. In 20 of the 1940 brandies stored in five-gallon containers the pH had decreased from an original average of 5.01 to 4.08 in 1942. The average original pH of 29 of the 1940 brandies stored in 10-gallon barrels was 4.87, but in 1942 it had dropped only to 4.21.

These differences in pH are closely paralleled by the changes in acidity. The reason for the slightly higher volatile acidity in the 50-gallon container compared with the 25 in the 1942 analysis is not apparent, since previously there was less volatile acidity in the brandy in the large container. It is possible that the bung on the 50-gallon barrel may have come loose during aging.

Pretreatment of the cooperage with sodium carbonate had a measurable effect, the pH of the brandy being higher than that stored in untreated cooperage. This was probably due to the neutralizing influence of the alkali on the wood since the barrels were very thoroughly washed with water after treatment. Rinsing and steaming the barrels one and a half hours and soaking them with brandy two or eight hours did not seem to make much difference in the pH changes. Used cooperage slowed down the pH change very materially.

In connection with this same study distillations at different proofs and from wines made of various varieties of grapes have been made. Neither the proof of distillation nor the variety of grape from which the distilling material was made appeared to have any influence on the total acidity or on the pH of the resulting brandy when cut and aged.

DISCUSSION

There is a lack of relationship between pH and titratable acidity in new brandies. Acetic acid is the predominant acid present and does, of course, have some buffer action. Schicktanz and Blaisdell (1940) have found the titration curve of aged whiskies to differ from that of young whisky, presumably because of the extraction of acidic substances from the wood during aging. The fact that the pH continues to decrease indicates that acetic acid is increasing since it has a pK lower than that of the other organic acids present.

The variability in the pH of newly distilled California brandies is remarkable. The excessive use of sulfur dioxide in the fermentation of the distilling material is reflected in the occasional low pH of new brandy and in the low pH of several of the aged brandies. The changes in pH in small cooperage are greater than that in large cooperage. The pH of sulfur dioxide-free brandies falls to approximately a pH of 4. It is not known whether this is true of all brandies. Crampton and Tolman (1908), how-

ever, found in whisky that the acid content approaches a maximum after three or four years in the wood. A similar equilibrium is apparently reached in brandy.

SUMMARY

The pH of 10 commercial, 19 newly distilled, and 84 experimental brandies was determined. Commercial brandies have a pH of 3.38 to 4.47 and average 4.10. Newly distilled brandies have pH's of 3.28 to 7.55, averaging 5.06. In the experimental brandies the pH decreased during aging. The decrease was larger and more rapid in small cooperage compared with large. Pretreatment of the barrel had a marked influence on the changes in pH if the barrel had been previously used or washed with sodium carbonate.

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STUDIES WITH BRANDY. II. TANNIN

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It is commonly assumed that there is an increase in the tannin content of brandies during aging although there is very little analytical data on the subject. Newly distilled spirits contain no coloring matter or tannin. During aging both are dissolved from the wood. The tannins extracted from various types of wood differ in their properties, those from oak being the most desirable for the aging of brandy. Oak from different locations is considered to be of varying degrees of desirability, and producers of high-quality brandy have marked preferences for containers made of oak from particular localities.

According to Russell (1935) the chief natural tannin occurring in oak wood is a phlobatannin. Phlobatannins are phlobaphene-producing tannins and phlobaphenes are red or brown, amorphous, insoluble materials which are most easily produced from the phlobatannins by heating with dilute mineral acids. The fission products of oak-wood tannin obtained by Russell were phloroglucinol and gallic acid. Freudenberg and Vollbrecht (1922), however, by means of tannase hydrolysis, obtained five per cent glucose, 23 to 25 per cent ellagic acid and amorphous quercusic acid. Kurmeier (1927) found ellagic acid but no sugar. Others have reported protocatechuic acid and catechin as oak-wood tannin fission products. This confused picture led Gisvold and Rogers (1939) to consider oak tannin as an unclassified tannin rather than a phlobatannin. (The confusion may be more apparent than real owing to the varying types and ages of oak wood which have been analyzed by various investigators.)

Whatever the exact constitution of oak tannin, it is chemically related more or less closely to catechin, and one of the fission products frequently reported from catechin as well as from oak tannins is protocatechuic acid. Vanillin is the methyl ether of protocatechuic acid.

Reif (1927) has reported the presence of 0 to .2 mg. of vanillin per liter of spirits. The vanillin he believed to have been extracted from the wood, and the amount found was roughly proportional to the brown color of the spirits. A perceptible vanillin-like odor is observed when brandy is first removed from a new oak barrel after aging for three or four years. Only very minute amounts of vanillin are necessary to give a detectable odor. Watts (1939) states that 2×10^{-10} mg. of vanillin per liter of air can be detected. There is a possibility that young red wines may contain vanillin and that it would be distilled. Seifert (1938) reports that Mach found vanillin in grape seeds and that red wines, which are fermented on the seeds, had a perceptible vanillin odor.

In general, it is known that the smaller the cooperage the more rapid the coloration and the faster the extraction of tannin. Probably this is because of the greater surface-to-volume ratio in the smaller containers and also because of the greater percentage loss of liquid from small containers

and resulting concentration of the nonvolatile constituents remaining. The longer the aging the greater the tannin content. Used cooperage obviously contains less extractable tannin material.

Joslyn [Joslyn and Amerine (1941)] made experiments on the effect of treatment of the cooperage on the tannin content. After one year of aging in 50-gallon barrels he found that brandy stored in barrels which had been treated with sodium hypochlorite, soda ash, or which had been steamed, paraffined, or charred before use, had a smaller tannin content compared with brandy stored in untreated new white oak containers. Addition of two ounces of quercyl seemed to increase the extracted tannin by the method of analysis used. Except for the marked reduction in tannin content after hypochlorite or soda ash treatments, the results were somewhat variable.

Valaer (1941) reported that the tannin content increases with age and that rapid-aging procedures involving the use of oak chips increases the tannin content disproportionately to the other constituents. He reports 22 mg. of tannin per 100 ml. in a brandy sample and 32 mg. in whisky. These figures may not be exact, since his reaction time did not exceed 15 minutes. Rosenblatt and Peluso (1941) found 22.7, 60.1, and 72.7 mg. per 100 ml. in three whiskies.

When brandies that have been stored in new oak cooperage are reduced in proof from 103° to 88°, a reddish precipitation frequently occurs. A portion of this precipitate is apparently due to the fact that caramel syrup becomes less soluble. Phlobaphene precipitation, however may also be partially responsible. Yasin (1938) has shown that extracts made from freshly cut oak settle slowly and are difficult to filter, while extracts from dry oak settle well and filter easily. The time of sedimentation, therefore, appears to be directly proportional to the moisture content of the wood and inversely proportional to the age of the wood. Improperly seasoned new oak has often been used for brandy in California. The tannin extracted from some of it is probably a cause of sediment in certain brandies.

EXPERIMENTAL PROCEDURE AND RESULTS

The tentative procedure of the Association of Official Agricultural Chemists, as proposed by Valaer (1941), was used for the determination of tannin except that the solutions were allowed to stand one hour before making any readings, as recommended by Rosenblatt and Peluso (1941). This method depends on the reactivity of the Folin-Denis reagent (made of sodium tungstate, phosphomolybdic, and phosphoric acids) for oxyphenyl compounds and is therefore not specific for tannins. Interfering substances are present only in small amounts in brandy. The tannic acid used contained eight per cent moisture and this was taken into account in the calculations.

An attempt was also made to measure the vanillin content. Reif's procedure (1927) was tried but only qualitative data could be obtained. A number of colorimetric procedures were investigated, but in all cases the same color or a similar color could be produced by tannin. Such colorimetric tests as bromine and ferrous sulfate, resorcinol, phloroglucinol, or ferric chloride all failed. Reif's sublimation procedure was also tried.

Small quantities of vanillin seem to be detected by this latter method, but quantitative data are difficult to obtain. It is concluded that if vanillin is present, it occurs only in very small amounts.

The color of the brandies was determined by the method previously reported by Tolbert and Amerine (1942). The standard contained 1.1, 1.0, and .2 ml. of one-half per cent solutions of red, yellow, and blue Eastman ABC dyes per 100 ml.

The brandies used for tannin determination are the same as those reported in the previous article by Guymon, Tolbert, and Amerine (1943).

The tannin and furfural content of the Exposition brandies are given (Table 1); the considerable variation in tannin content between the samples indicated that they had been aged for different periods of time or that oak chips had been used. We have been advised that this was the case for the two brandies containing the largest amount of tannin. In general the high tannin samples had high furfural, extract, and total acid.

TABLE 1

Tannin, Furfural, Total Acid, and Extract Content of Exposition Brandies

No.	Tannin	Furfural	Total acid	Extract
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
A	10.2	0.27	.0322	.0984
B	9.3	0.24	.0276	.0968
C	17.0	1.04	.0480	.1306
D	49.2	1.89	.0575	.2342
E	3.0	0.94	.0390	.0266
F	10.0	0.19	.0193	.0740
G	10.6	0.43	.0257	.1294
H	47.5	1.00	.0515	.1820
I	20.5	1.02	.0658	.1486
J	16.5	0.79	.0377	.1410

No relationship between the amount of tannin and the ester, fusel oil, aldehyde, acetal, copper, or ash content was observed.

The Prorate brandies were newly distilled and very recently barreled when the samples were taken. Very little tannin could therefore be found, only a trace to 2.0 mg. per 100 ml., averaging only 1.0. This is probably not all tannin, small amounts of other substances reacting with the Folin-Denis reagent being present.

The pertinent analytical data on the University brandies are given (Table 2); the data have also been recalculated on the basis of the amount of tannin per 100 ml. of original brandy present. The tannin and color content increase with age, are greater in small than in large cooperage, and do not appear to be affected by moderate pretreatment of the cooperage except after actual brandy storage or after charring and scraping. There is a very marked reduction in color and tannin extraction in these two latter cases. In the 50-gallon barrel there was about 30 mg. of tannin per 100 ml. after two years of aging.

Although there is a regular progression in tannin content in the barrels of different sizes after one year of storage, the 50-gallon container is inconsistent in the second and third year. When this container is finally

TABLE 2

Tannin and Color Content of University Brandies in Various Types of Containers

University No.	Number of containers	Barrel size [1]	Barrel treatment [2]	Tannin: To original volume 1941 [3]	Tannin: To original volume 1942	Tannin: To final volume 1941	Tannin: To final volume 1942	Color 1941	Color 1942
			1939 Brandies						
				mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*		
17	1	5	Rinsed with brandy	32.0	33.6	38.0	51.5	73	87
18	1	10	Rinsed with brandy	28.7	30.2	32.6	41.7	39	51
19	1	15	Rinsed with brandy	23.1	26.6	25.2	32.8	36	48
20	1	25	Rinsed with brandy	23.8	26.4	25.4	30.8	40	52
21	1	50	Rinsed with brandy	22.6	28.6	23.5	32.1	52	73
22	1	50	Charred; scraped out; rinsed with brandy	6.7	9.6	7.1	11.0	12	16
23	1	25	Two-year-old brandy barrel	7.3	11.2	8.1	13.7	22	30
27	1	5	Na_2CO_3 wash; soaked with brandy	36.2	38.0	41.7	56.2	64	83
....	19	5	Rinsed with brandy	37.1	38.5	44.0	57.5	66	80
....	3	5	Steamed; soaked with brandy	33.5	36.8	39.4	56.7	65	81
			1940 Brandies						
78	1	25	Steamed; soaked with brandy	13.2	19.3	14.9	24.0	30	53
79	1	25	Old brandy barrel	5.4	10.9	5.9	13.4	14	25
....	22	5	Boiled hot water; soaked with brandy	33.6	37.5	38.3	55.8	75	122
....	29	10	Boiled hot water; soaked with brandy	20.0	26.0	22.6	35.0	32	55

[1] In gallons. [2] All barrels were new unless otherwise stated. [3] Actual dates of withdrawal of samples: 1939 brandies were put in barrels Dec. 22, 1939, to Jan. 16, 1940; 1941 analyses were drawn Jan. 15, 1941; 1942 analyses on Jan. 19, 1942; 1940 brandies were put in barrels Oct. 23, 1940, to Jan. 16, 1941; 1941 analyses were drawn Aug. 4, 1941; and 1942 analyses on Aug. 8, 1942.

emptied it will be possible to examine the inner surfaces of the staves to determine if any faults were present which could account for the greater color and tannin extraction.

DISCUSSION

The amounts of tannin present in California brandies are decidedly variable. This is one of the reasons for the dissimilarity of many of the commercial brandies. The differences arise from the use of new oak *versus* used cooperage, from differences in temperature and period of storage, and from the use by some producers of oak chips or tannin extracts. The presence of too much tannin in brandy is also reflected in too dark a color. These dark brandies have an astringent or harsh taste which is undesirable. The tannin content may be readily reduced by the use of certain types of activated charcoal, according to Tolbert and Amerine (1942).

TABLE 3

Tannin Extracted From Oak Barrels[1] per Square Sentimeter of Exposed Surface per Year

University No.	Approximate barrel size	Area[2]	Tannin extracted[3]		
			1941	1942	Total
	gal.	*cm.²*	*mg./cm.²*	*mg./cm.²*	*mg./cm.²*
17	5	3,965	1.47	.08	1.55
18	10	6,200	1.69	.09	1.78
19	15	9,100	1.45	.20	1.65
20	25	12,200	1.86	.20	2.06
21	50	18,440	2.22	.59	2.81

[1] New barrels were rinsed with brandy only before use. [2] Area equals:

$$\frac{\pi}{2}\left[\sqrt{(b^2-d^2)+h^2d^2}+\frac{h^2b^2}{\sqrt{(b^2-d^2)(h^2+d^2-b^2)}}\quad \sin^{-1}\quad \frac{\sqrt{(b^2-d^2)(h^2+d^2-b^2)}}{bh}\right]+\frac{\pi}{2}d^2$$

where h = depth, b = diameter at center, d = diameter at the top and bottom (all inside measurements). The authors are indebted to Professor E. B. Roessler for this formula. [3] Calculated to the original volume of the brandy.

The increase in tannin during aging occurs in much the same fashion as in whisky, although the amounts found in brandy are somewhat greater. The amounts extracted per square centimeter of exposed surface per year in containers of different sizes are given (Table 3).

The amount of tannin extracted by brandies contained in small cooperage is much greater in concentration than by those contained in large cooperage (Table 2). However, it appears from Table 3 that there is a lesser degree of extraction of the total tannin from the wood by brandies stored in small cooperage. This may be due to the fact that the smaller containers have a smaller percentage of their available surface in contact with the brandy after one or two years of aging. But as more total tannin is extracted after the first year of aging from the largest barrel, the extraction must be more dependent upon the concentration of tannin and solids in the brandy. In agreement with this the rate of tannin extraction is greatest during the first year.

The rate of extraction of tannin by brandies is therefore assumed to be limited by the concentration of tannin, and possibly of other soluble material in the brandy. The total tannin extracted from the five- and 10-gallon

barrels after two years of aging is only about one-third as much as in the 15- and 25-gallon containers. The only other apparent explanation is that the tannin has been removed from the staves of the smaller containers; but Table 3 indicates that this is not the case, since more tannin is extracted per square centimeter from the large containers which are made from the same type and age of wood.

The color differences are roughly the same as the tannin differences. Crampton and Tolman (1908) found a gradual darkening of whiskey. Valaer (1941), however, in his studies on the aging of brandy did not find a very regular increase in color.

SUMMARY

The tannin content of commercial, newly distilled, and experimental brandies has been determined. Commercial brandies contain from three to 47.5 mg. of tannin per 100 ml., averaging 19.3 mg. Newly distilled cut and colored brandies contain only about one mg. per 100 ml. of tannin as determined with the Folin-Denis reagent. The tannin, furfural, total acid, extract, and color contents increase more or less at the same rate. Brandy stored in small cooperage contains more tannin than that stored in large cooperage. This is true even if the losses owing to evaporation are taken into account. However, more tannin is extracted per square centimeter of surface from larger barrels. The concentration of tannins and other solids in the brandy is apparently the limiting factor for the amount of tannin extracted from the barrel. Very little vanillin could be found in California brandy.

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ASCORBIC ACID CONTENT OF COW'S MILK DURING FOUR SUCCESSIVE LACTATION PERIODS

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In earlier papers the authors (1939, 1940a, 1940b, 1941, 1942) have discussed the influence of breed, seasonal variation, pasteurization, age of the cow, the stage of lactation, pregnancy, and the daily volume of milk produced upon the ascorbic acid content of cow's milk. The relationship of the stage of lactation to the ascorbic acid content of milk has also received attention by Rasmussen, Guerrant, Shaw, Welch, and Bechdel (1936), Whitnah and Riddell (1937), and Reedman (1937), but a survey of the literature revealed no data concerning the ascorbic acid content of milk from successive lactations. During the study of the various factors noted above upon the ascorbic acid content of milk, it was frequently necessary to replace the cows employed in the study. One cow which produced milk with a high vitamin C content was used to compare the ascorbic acid content of milk produced under comparable conditions during successive lactations.

EXPERIMENTAL PROCEDURE

The cow used in this study, a grade Guernsey, was born and raised in Nova Scotia and shipped to Massachusetts after she was in production. On arrival at the farm she was subjected to a 60-day quarantine to eliminate any possibility of her being affected with mastitis, infectious abortion, or other contagious bovine disease. Throughout the experiment the cow weighed about 880 pounds and was in sound physical condition. During the four years that this cow was under observation she was maintained under uniform housing and feeding conditions. She was a member of a herd maintained for the production of certified milk, was housed in a modern dairy barn, and was continuously under veterinary supervision. Throughout the four years she was continuously stall fed and did not have access to pasture. At all seasons of the year the cow was fed a well-balanced dairy ration which consisted of mixed hay, grass ensilage, beet pulp, and grain mixtures. The various components of the ration were described in detail in an earlier paper (1939) of this series which also reported the ascorbic acid content of typical components of the ration.

The present study of the ascorbic acid content of cow's milk during successive lactation periods is concerned with the third, fourth, fifth, and sixth lactation periods. At the beginning of the experiment the cow was

in the 14th month of her third lactation period. The ascorbic acid value of her milk was determined for five successive months or until the 19th month of lactation. After 19 months of continuous lactation the cow was given a rest period from about the first of January to the middle of August when her fourth calf was born. Assays of the vitamin C content of her milk were again conducted during the first six months of the fourth lactation. The fifth calf was born 13 months after the beginning of the fourth lactation, and the vitamin C content of the milk was determined during the fifth, sixth, seventh, eighth, and ninth months of the fifth lactation period. The sixth calf was born a little over 13 months after the birth of the fifth calf. During the sixth lactation period the vitamin C content of the milk was studied from the second to the sixth months of lactation, inclusive. At this time the cow was 11 or more years old but still in excellent physical condition.

Samples of milk for ascorbic acid assay were collected at the morning milking about 3:30 A.M. They were immediately placed in one-ounce flint glass bottles which were rapidly cooled and stored on ice out of contact with light until they were assayed six to 12 hours later. The ascorbic acid content was determined by the indophenol titration method of Tillmans as modified by Bessey and King (1933). The procedure followed in determining the ascorbic acid content of the milk has been previously described in detail by Tripp, Satterfield, and Holmes (1937).

The results of the study (Table 1) include data concerning the stage of lactation, the amount of ascorbic acid per liter, the amount of milk produced per day, and the total ascorbic acid production per day. In the third lactation period the ascorbic acid content of the milk varied from a maximum of 31.37 mg. of ascorbic acid per liter during the 16th month to a minimum of 22.74 mg. per liter at the 19th month of lactation.

At the beginning of the fourth lactation period the ascorbic acid content of the milk, 16.8 mg. per liter, was the lowest that it was at any time during the four-year study. The previous, unusually long, 19 months' lactation period can hardly have been the cause since in the interim there was a long, seven months', nonlactating rest period. The ascorbic acid content of the milk rose steadily to 25.32 mg. at the fourth month of lactation and remained nearly as high during the fifth and sixth months of lactation.

During the fifth lactation period the milk was assayed from the fifth to the ninth months, inclusive. The ascorbic acid content of the milk showed considerable variation, 19.62 to 23.97 mg. per liter, during the eighth month of the lactation period.

In the sixth lactation period ascorbic acid assays of the milk were made during the second to the sixth months, inclusive. The lowest ascorbic acid content, 18.68 mg. per liter, occurred at the end of the second month and the maximum content, 23.80 mg. per liter, occurred at the beginning of the second month. These results are similar to those obtained in the fifth lactation period. The ascorbic acid content of the milk from the different lactation periods may be easily compared by referring to Fig. 1. The average ascorbic acid content per liter of milk was 26.93 mg. for the 14th to the 19th months of the third lactation, 22.10 mg. for the first to the

TABLE 1

Ascorbic Acid Content of Cow's Milk During Successive Lactation Periods

Month of lactation	Daily milk production	Ascorbic acid per liter	Total daily ascorbic acid production
	liters	*mg.*	*mg.*
THIRD LACTATION PERIOD			
14	10.52	24.63	259
15	8.33	29.81	249
16	8.41	25.80	217
16	7.09	31.37	222
17	7.84	27.50	215
17	8.36	25.70	215
17	8.01	27.10	217
18	7.93	27.03	214
18	6.60	27.68	183
19	6.65	22.74	150
Average	7.97	26.93	214
FOURTH LACTATION PERIOD			
1	16.20	16.80	272
1	20.08	19.28	386
2	18.14	21.49	389
3	18.45	22.77	420
4	15.85	25.32	402
5	15.19	24.27	368
6	14.71	24.78	364
Average	16.94	22.10	372
FIFTH LACTATION PERIOD			
5	16.24	19.89	322
5	12.33	21.60	266
5	12.24	22.83	279
6	12.33	22.66	278
6	11.93	22.16	264
7	11.67	21.39	249
7	11.36	21.60	246
8	9.60	23.97	230
8	7.53	19.62	148
9	4.84	22.07	107
Average	11.00	21.78	239
SIXTH LACTATION PERIOD			
2	22.02	23.80	524
2	20.26	18.68	378
3	19.51	23.47	458
3	19.20	19.13	367
3	19.06	22.53	430
4	20.03	22.34	448
4	20.52	22.63	464
5	18.54	20.87	387
5	18.02	23.25	419
6	20.17	22.18	447
Average	19.73	21.89	432

sixth months of the fourth lactation, 21.78 mg. for the fifth to the ninth months of the fifth lactation, and 21.89 mg. for the second to the sixth months of the sixth lactation.

The daily volume of milk produced in the third lactation period decreased from 10.52 liters at the 14th month to 6.60 liters at the end of the 18th month and averaged 7.97 liters. During the first six months of the fourth lactation the volume of milk decreased from 20.08 to 14.71 liters

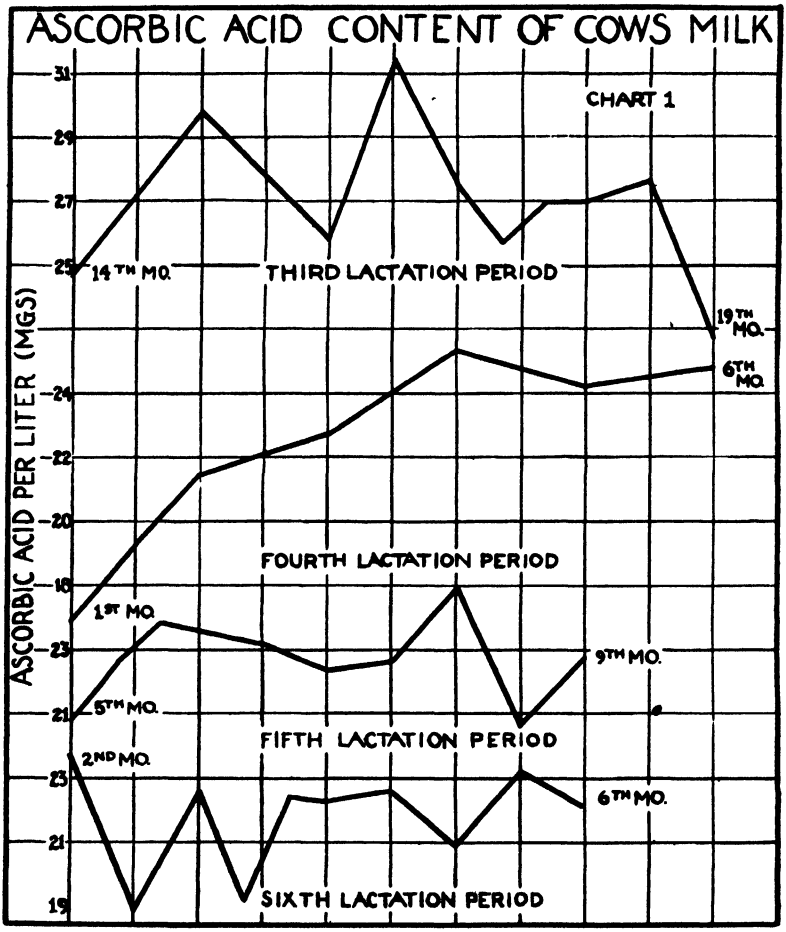

FIG. 1.

and averaged 16.94 liters. In the fifth lactation the decrease was from 16.24 to 4.84 liters with an average of 11 liters. The fluctuation in milk volume during the sixth lactation was from 22.02 liters in the second month to 18.02 liters in the fifth month with an average of 19.73 liters.

Obviously the total daily ascorbic acid production was influenced by both the volume of milk and its ascorbic acid content. From the 14th month of the third lactation period the daily ascorbic acid production decreased from 259 to 150 mg. at the 19th month. The total ascorbic acid

production increased in the fourth lactation period from 272 mg. at the beginning of the first month of lactation to 420 mg. in the third month of lactation. In the fifth lactation period the daily production of 322 mg. of ascorbic acid at the fifth month decreased to 107 mg. at the ninth month of lactation. The maximum daily ascorbic acid production for the sixth lactation period, 524 mg., came in the second month and the minimum, 367 mg., occurred at the third month of lactation. The daily ascorbic acid was quite high, however, for the entire first six months of the sixth lactation period. The average daily ascorbic acid production was 214 mg. in the third lactation, 372 mg. in the fourth lactation, 239 mg. in the fifth lactation, and 432 mg. in the sixth lactation. Quite likely the relatively lower production in the third lactation was due to the long lactation period. However, the ascorbic acid production in later lactations, particularly 432 mg. in the sixth lactation when the cow was 11 years old, would indicate that the ability of this Guernsey cow to produce milk rich in ascorbic acid had not diminished with advancing years or with successive lactations.

SUMMARY

A study has been made of the ascorbic acid content of milk produced under uniform conditions by a grade Guernsey cow during her third, fourth, fifth, and sixth lactation periods. The stages of lactation were from the 14th to the 19th months, inclusive, from the first to the sixth months, from the fifth to the ninth months, and from the second to the sixth months, respectively, of the four lactation periods.

The average volumes of milk produced daily were 7.97, 16.94, 11, and 19.73 liters, respectively. The ascorbic acid content of the milk varied for the individual assays but averaged 26.93, 22.10, 21.78, and 21.89 mg. per liter, respectively, for the four lactation periods. The total daily ascorbic acid production averaged 214, 372, 239, and 432 mg. per liter, respectively, for the third, fourth, fifth, and sixth lactations. Thus the ability of this cow to produce milk rich in ascorbic acid did not diminish with advancing years and successive lactations, even though she was 11 years or more old at the conclusion of the experiment.

ACKNOWLEDGMENT

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COMPARISON OF THE DOLMAN KITTEN TEST, THE STONE CULTURAL SCREEN TEST, AND THE SLOCUM-LINDEN AGGLUTINATION TEST FOR ENTEROTOXIC STAPHYLOCOCCI

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The final test for the presence of bacterial toxins is animal injection. However, animal injection will not always detect toxin producers since toxin production is a variable factor depending to a considerable extent upon environment. Also, test animals may be expensive or seasonal and may vary in susceptibility. For these reasons any substitute for animal injection is welcome. Other distinctive characters, if retained independently of the toxin-producing factor, would be useful in detecting potential toxin producers, although isolation from food of such organisms may lead to error in diagnosis. Stone (1935, 1939) proposed a physiological test for the detection of enterotoxic staphylococci and reported a high degree of correlation with the Dolman kitten test. Other workers failed to obtain the same close correlation. Slocum and Linden (1939) reported on a normal horse serum-agglutination test which, if of value, further simplifies the detection of enterotoxic staphylococci. It is the purpose of this paper to report on a comparison of these three tests.

Nineteen strains of staphylococci isolated from food-poisoning outbreaks were obtained from various sources. Some of these strains were reported as giving positive tests for enterotoxic staphylococci by one or more methods. These tests were made in different laboratories by workers who varied greatly in experience and skill. On this account and the fact that other organisms may possibly be responsible for poisoning symptoms very similar to those caused by staphylococci—Cooper, Davis, and Wiseman (1941)—we could not be certain that the strains reported as enterotoxic were toxin producers.

EXPERIMENTAL PROCEDURE

The 19 strains stored for different periods on a beef-heart medium were tested one or more times by the three methods. In applying the Dolman and Wilson (1938, 1940) kitten test, at first their method was used but later the modification recommended by Phatak and Pentler (1940). Kittens were used mainly, but adult cats were found to be as satisfactory. Some of the tests were made on kittens previously used once, but usually animals were used only once since some tolerance developed when repeated injections were given.

Favorite and Hammon (1941) described a special medium they found useful in studying toxin production by a few strains, and Hammon (1941) recommends intravenous injection. We did not find the Favorite and Hammon medium as satisfactory as the Dolman and Wilson medium used, and Bayliss (1940) did not note any superiority in the intravenous method of injection.

In interpreting the Stone test we used a chart, submitted by Dr. Stone, showing the different sized zones of liquefaction as a standard. Clear zones with a radius of .4 centimeter between edge of colony and edge of zone were considered positive. Pigmentation was determined after three days' incubation by taking a loopful of a colony and examining it for color. Some tests recorded as positive by this method would have been recorded as negative if the colonies had been viewed directly on the Stone or blood-agar medium.

The strains were classified according to the Stone test as follows:

	Stone reaction	Hemolysis	Pigment
Stone positive	+	—	+
Stone positive	+	+	—
Stone positive	+	+	+
Stone negative	—	+	+
Stone negative	+	—	—

The Slocum and Linden (1939) test is based on a difference in agglutinability between enterotoxic and non-enterotoxic staphylococci in normal horse serum. We at first used their method as described and then changed to the following procedure:

Growth on 24-hour slants was washed off with physiological saline and filtered through cotton. Suspensions were adjusted to a turbidity equal to Tube 3 of the McFarland nephelometer. Sera were obtained from five normal horses. Equal amounts of the bacterial suspension and serum dilutions up to 1:640 were placed in tubes and incubated for 24 hours. In tabulating results we used 1:80 as a critical serum dilution and the average obtained with the five sera as a unit.

DIFFICULTIES AND PRECAUTIONS TO BE OBSERVED IN APPLYING TESTS

In applying the Dolman kitten test six c.c. of filtrate per kilo of body weight were injected and never more than eight c.c. regardless of weight. Animals were turned over each hour. Observation continued for 24 hours. It was found that emesis may be delayed for as long as 18 hours. Emesis occurring in less than 20 minutes should be regarded as nonspecific. Excessive doses of nontoxic filtrate may cause emesis under 20 minutes—Minett (1938) and Pentler (1940). Presence of non-enterotoxic toxins and septicemia may also lead to emesis.

When kitten symptoms are typical, as described by Dolman and Wilson (1940), Pentler (1940), and Bayliss (1940), diagnosis is simple; but symptoms in positive cases are not always typical and require interpretation. Diarrhea and urination may follow injection of uninoculated medium and should be ignored. Also, licking of lips, quivering of hind legs, abdominal paroxysms or contractions alone should not be considered positive. Animals vary in susceptibility to the same filtrate. With doubtful and negative reactions, the test should be repeated with different animals.

One of the greatest difficulties associated with the kitten test is that toxin production is a variable attribute. Storage on media frequently

leads to loss of toxin production, Slocum and Linden (1939). Our strains were stored on a beef-heart medium for over a year before the tests were made. Transfers from this medium to the Dolman and Wilson medium were made for toxin production. Since we obtained more positive strains than reported at isolation, the beef-heart medium may have value in the maintenance of toxin production.

The Stone test presents fewer difficulties than the kitten test; however, the degree of variation in the three characteristics utilized is not so well known. This test has one advantage over the other two tests, it has only one living organism involved in addition to the technician.

The difficulties of the agglutination test revolve around the variations in agglutinins in normal horse sera. Such a test, to be satisfactory, requires that all normal horse sera contain stronger agglutinins for the non-enterotoxic strains or that a blood supply be carefully selected. Our results indicate that great variation occurs in the agglutinating power of normal horse sera. The sera of some horses will be of no value in this test unless a standard is determined for such animals.

We averaged the titers obtained with sera from five horses and used a dilution of 1:80 as the critical dilution. No other dilution increased the agreement with the kitten tests. Some of the kitten negative strains failed to agglutinate in this dilution and this accounted for most of the disagreement with the kitten test.

COMPARISON OF RESULTS

The Stone test gave us a 65-per cent agreement with the kitten test. This was slightly less than the 77-per cent agreement reported by others at time of isolation.

Our results and experience suggest that the Stone test may give more uniform results in the hands of different or inexperienced workers than the kitten test. When the Stone test gives a positive reaction with a kitten negative strain, it is not possible to state which is accurate. The final test for toxin, but not toxin producers, is the effect on an animal.

At isolation of our 19 strains the Stone test gave more positive reactions than the kitten test. As conducted, the Stone test also produced more positive reactions than the kitten test. Storage on media has been reported as affecting both tests. One test may possibly be affected independently of the other.

The Slocum and Linden agglutination test as modified gave an agreement of 69 per cent with the kitten test, approximately the same as obtained with the Stone test. Figured separately, the five sera gave agreements varying from 47 to 73 per cent.

If the agglutination test is to be used, a group of five or more horse sera should be used, as in these experiments, until a suitable supply from a single animal is obtained. The value of the agglutination test remains to be determined.

The value of comparing results obtained with the Stone and Slocum and Linden tests with those obtained with the kitten test is questionable. It is not logical to attempt to determine the value of a test by using an

obviously defective test as a standard, Singer and Hagan (1941). The kitten test must be greatly improved before we can accept it as a standard for determining the value of other tests. It is based on the presence of toxin, not on the ability to produce or to acquire the ability to produce toxin. The correlation between toxin production and other characteristics of toxin producers is not absolute. Favorite and Hammon (1941), in a study of three strains, found the correlation between toxin production and hemolysis variable. The use of these other characteristics in detecting toxin producers or potential toxin producers is defensible. The kitten test has not been standardized, and its accuracy will vary with the worker as well as with the organism and the kitten. Attempts to improve the test should be directed toward preventing variation in toxin production and more accurate interpretation of the effect on kittens. It requires more skill in application as more variable factors are involved.

DISCUSSION

The frequency of staphylococcus food poisoning suggests that conditions favorable for toxin production commonly occur. Since those conditions, once they become known, can undoubtedly be easily duplicated, the question naturally arises whether testing for toxin production under highly specialized and unnatural conditions may not lead to error in diagnosis. Tests may become too sensitive, and strains not producing toxin under conditions occurring in eating establishments may do so in the laboratory. Staphylococci are usually present in carelessly handled food. The isolation of such an organism when few are present and testing for toxin production under unnatural conditions, introduces a possible source of error.

Toxin production may depend on either the immediate environment, the past history of the strain, or chance variation in what appears to be a uniform environment. When the latter occurs, selection of colonies becomes a determining factor in testing for toxin production. Haberman and Miller (1942) studied 137 variants from seven parent cultures. Most of the variants lost toxin production but others gained it. The factor determining these changes is unknown.

Various authors have reported isolating enterotoxic staphylococci from cases of mastitis, the noses and throats of food handlers, and other human infections, Chapman (1941). Contact with living tissue in the immediate past history of the strain may be one of the determining factors contributing to toxin production. Slanetz (1942) obtained "beta toxins" from 96 of 105 strains isolated from cases of mastitis. His toxin produced positive kitten test. No one has, as far as we know, attempted to enhance toxin production by inserting culture in the cow's udder. Our attempts to enhance toxin production by growth in the developing chick embryo, blood, milk, and egg were unsuccessful. Search for a more favorable medium should continue, but we should not lose sight of the possibility that use of such a medium may introduce a source of error by stimulating toxin production in a strain not producing it under conditions prevailing in eating establishments.

SUMMARY AND CONCLUSIONS

The Dolman and Wilson kitten test, the Stone cultural screen test, and the Slocum and Linden agglutination test, somewhat modified, were made on 19 strains of staphylococci isolated from food suspected of causing staphylococcus food poisoning. Comparison of results is given and the defects of the tests are discussed.

Comparison of other tests with the kitten test cannot be of much value in determining the accuracy of the former until the kitten test is standardized and its accuracy demonstrated more fully than at present.

The Stone test (65 per cent) and the Slocum and Linden test (69 per cent) gave approximately the same agreement with the kitten test.

Variations in the living agents, the organisms, the kittens, the horses, and the technicians interfere with the tests.

Since the kitten test gives variable results as at present applied, it is not advisable to rely solely upon it for the detection of enterotoxic staphylococci. The significance of a positive Stone or Slocum and Linden test on kitten negative strains remains to be determined. Until the kitten test and toxin production are better controlled or replaced by a more accurate method, it appears advisable to consider such strains as probably enterotoxic.

Since all qualitative tests are at present unsatisfactory, it is desirable to make a quantitative test for supporting evidence in food-poisoning outbreaks. The relation between numbers of organisms and toxin production should be determined. Toxin production probably does not occur without abundant growth according to Segalove and Dack (1941). The isolation of a toxin-producing strain from infected food should not result in the condemnation of such food unless staphylococci are present in considerable number. In the examination of foods a positive test for the presence of toxin is more significant than the isolation of toxin producers.

Of 19 strains isolated from food suspected of causing staphylococcus food poisoning, six were positive by the kitten test, eight by the Stone cultural test, and eight by the agglutination test. Applying all three tests, 15 of the strains were positive. Controls of stock strains of *Staphylococcus aureus* and *citreus* were negative to all tests as were four of the 19 strains studied.

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DETERMINATION OF TOUGHNESS OF FROZEN ASPARAGUS *(ASPARAGUS OFFICINALIS)*[1]

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The determination of alcohol-insoluble solids has been used to ascertain the maturity of starchy vegetables by Kertesz (1935a,b), Lee (1941), and Lee and DeFelice (1942), but it has not been proposed as an index of quality for the nonstarchy type.

Although most freshly harvested asparagus is tender, rather tough spears have been cut fresh from the field. Furthermore, this vegetable tends to toughen on standing after cutting, especially when the spears are not stored under the most favorable conditions. Inasmuch as toughness is one of the important factors to be considered in determining the quality of this product, it is desirable to have available methods for the accurate estimation of this character of the frozen product that are independent of personal judgment. The toughness of the fresh vegetable can be determined by means of the tenderometer, as shown by Jenkins and Lee (1940).

Frozen asparagus is usually found on the market in five-inch spears graded according to the Agricultural Marketing Administration (1942) as small, medium, and large, depending on the diameter of the stalks. It can also be bought as fancy cuts, which product is a mixture of cut spears of any diameter. Spears cut too short in the factory cutting machine are further segmented and put into this grade. Many manufacturers put all spears with the small diameter into this grade. Soup cuts consist of the inch-and-a-half segments immediately below the upper five inches of the shoots, and are not sold in the retail trade.

The changes in the composition and rate of growth along the developing stem of this vegetable have been studied by Culpepper and Moon (1939).

EXPERIMENTAL PROCEDURE

The samples used in this study were graded as small, medium, and large and were cut to approximately five-inch lengths. The small and medium spears were blanched three minutes at 100°C.(212°F.). The large spears were blanched for three and one-half minutes at this same temperature. (This is standard commercial practice.) Following this they were packed so that duplicate boxes would contain the same material and were frozen and stored under commercial conditions. Some lots were obtained directly from the growers so that a complete history of each could be had. These were graded, treated, packed, and frozen as were the others. The remainders of each of these samples were stored at room temperature. Samples were taken at daily intervals, prepared, and frozen as before,

[1]Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 527.

until considerable spoilage was noticed. Other lots were made up of shoots that had been left in the field until high growth and much branching resulted. The upper five inches of these shoots were found to be very tender, not only as judged by the alcohol-insoluble substances but also by means of the tenderometer and the organoleptic tests. They were ungraded as to size. The second and third five-inch sections of these shoots were tested and likewise were ungraded as to size. These last three types of samples were blanched, packed, and frozen as before.

In conducting the analyses, samples were taken out of cold storage and allowed to thaw overnight at room temperature. They were drained for two minutes on a standard eight-mesh, eight-inch sieve. The spears were then cut into quarter-inch lengths and ground in a mortar. The material in the mortar was thoroughly mixed before each sample was taken out. Each 25-gram sample was washed into a 500-ml. pyrex Erlenmeyer flask with 300 ml. of 80-per cent ethyl alcohol and slowly boiled under a reflux condenser on a steam bath for 30 minutes. The samples were then filtered with suction while hot, using tared filter paper in the Büchner funnels, taking care to keep the mixture on the paper from going dry during filtration. After the insoluble matter was washed several times until white with hot 80-per cent ethyl alcohol, the paper and solids were placed in tared dishes and dried in an oven at 95°C.(203°F.) overnight. The weight of the paper and dish were subtracted from the total weight.

DISCUSSION

This study was made in 1940 and repeated in 1941, but the results of the latter year only are herewith presented in order to avoid duplication of similar data. The asparagus used for this work was the Mary Washington variety, the variety most commonly grown in New York state for freezing. The analytical method used is empirical, and is based on the standard five-inch length, the length that is frozen commercially.

The coefficient of correlation between alcohol-insoluble substances and the organoleptic tests for toughness is $-.8054 \pm .0348$ (standard error). It can be readily seen (Table 1) that as the asparagus gets tougher the alcohol-insoluble substances decrease. This trend is opposite from that in the starchy vegetables, such as peas and corn, in which alcohol-insoluble solids increase with advancing maturity.

The entire problem of quality in the case of asparagus is different from either corn or peas. Asparagus is harvested when the spears reach a height of seven to 10 inches from the ground and, consequently, does not have the opportunity to get old in the sense that peas and corn might. It is necessary that asparagus be harvested at this height for when it is allowed to remain in the field it branches and thus becomes unmarketable. Asparagus that is tough when harvested is so probably because of soil, climate, or other factors. It is interesting to note that while increase in toughness is in general proportional to the length of time in room-temperature storage, other factors, such as the temperature of the room and source of the asparagus, may influence toughening so as to retard it materially.

A study of the table further shows that the alcohol-insoluble substances are higher in most of the samples of fancy cuts than in the spears. This

TABLE 1

Results of Toughness Tests on Asparagus

Date 1941	Size [1]	Soil type [2]	Storage [3] (if any)	A.I.S.[4]	Predicted grade [5]	Organoleptic [6]	Remarks
				pct.			
6/2	Ung.		Fresh	6.50	....	1	Branched heads
6/2	Ung.		Fresh	5.99	....	1	Branched heads
6/11	F.C.		Fresh	5.83	F	1	
5/23	F.C.		Fresh	5.68	F	2	
5/26	F.C.		Fresh	5.61	F	1	
5/21	F.C.		Fresh	5.53	F	1	
6/13	F.C.		Fresh	5.44	F	2	
6/2	F.C.		Fresh	5.37	F	1	
5/14	F.C.		Fresh	5.37	F	2	
5/22	M		Fresh	5.35	F	1	
5/22	F.C.		Fresh	5.34	F	1	
5/12	S	River bottom	Fresh	5.32	....	1	
5/21	M		Fresh	5.19	F	1	
5/16	F.C.		Fresh	5.17	F	2	
5/23	M		Fresh	5.11	F	2	
5/28	M		Fresh	5.06	F	2	
5/22	M		Fresh	5.04	F	1	
5/23	M		Fresh	5.03	F	2	
5/19	S	River bottom	Fresh	5.00	....	1	
5/28	F.C.		Fresh	4.98	F	2	
5/26	M		Fresh	4.97	....	1	
5/16	M		Fresh	4.96	F	1	
5/12	S	Sandy	Fresh	4.93	....	1	
5/12	M		Fresh	4.92	F	2	
6/11	M		Fresh	4.90	F	1	
5/19	F.C.		Fresh	4.89	F	1	
5/26	M		Fresh	4.87	F	1	
5/23	M		Fresh	4.86	F	1	
5/28	M		Fresh	4.84	F	2	
6/2	M		Fresh	4.84	F	2	
6/2	M	River bottom	Fresh	4.84	....	1	
6/2	Ung.		Fresh	4.83	....	2	
5/19	M		Fresh	4.83	F	2	
5/22	L		Fresh	4.81	F	1	
5/26	M		Fresh	4.81	F	2	
5/21	L		Fresh	4.79	F	2	
5/21	M		Fresh	4.79	F	2	
5/12	M	Sandy	Fresh	4.76	....	1	
5/26	M		Fresh	4.76	F	1	
5/16	M		Fresh	4.76	F	1	
5/12	M	River bottom	24 hr.	4.74	....	2	
6/11	M		Fresh	4.72	F	1	
5/22	M		Fresh	4.71	F	3	
6/13	M		Fresh	4.66	F	1	
5/28	M		Fresh	4.66	F	3	
5/21	M		Fresh	4.66	F	3	
5/23	L		Fresh	4.64	F	1	
5/26	L		Fresh	4.64	F	2	
5/19	M		Fresh	4.62	F	2	
5/19	L	River bottom	Fresh	4.62	....	1	
5/28	M		Fresh	4.61	F	2	
5/16	L		Fresh	4.60	F	1	
5/16	M		Fresh	4.59	F	2	
5/12	L	River bottom	Fresh	4.58	....	2	
5/19	M		Fresh	4.57	F	2	
5/26	L	River bottom	Fresh	4.56	....	2	

TABLE 1 *(Concluded)*

Date 1941	Size [1]	Soil type [2]	Storage [3] (if any)	A.I.S. [4]	Predicted grade [5]	Organoleptic [6]	Remarks
				pct.			
5/14	L		Fresh	4.53	F	2	
6/11	L		Fresh	4.53	F	2	
5/19	M	River bottom	24 hr.	4.52	....	2	
5/19	M	River bottom	Fresh	4.51	....	2	
5/21	M	Muck	Fresh	4.50	....	2	
6/2	L		Fresh	4.48	F	3	
5/16	S	River bottom	72 hr.	4.48	....	2	
5/12	M	Sandy	48 hr.	4.44	....	3	
5/21	S	Muck	Fresh	4.44	....	3	
6/13	L		Fresh	4.42	....	4	
5/12	S	Sandy	72 hr.	4.40	....	2	
5/16	M	River bottom	24 hr.	4.39	....	3	
5/12	S	River bottom	72 hr.	4.38	....	4	
6/11	M		Fresh	4.36	F	1	
5/19	L		Fresh	4.35	F	2	
5/12	M	River bottom	72 hr.	4.33	....	3	
5/12	L	Sandy	Fresh	4.32	....	2	
5/12	M	River bottom	48 hr.	4.29	....	4	
5/12	M	Sandy	72 hr.	4.28	....	3	
5/12	M	Sandy	96 hr.	4.21	....	5	
5/21	L	Muck	Fresh	4.10	....	3	
5/12	L	Sandy	72 hr.	4.07	....	4	
5/19	M	River bottom	48 hr.	4.05	....	3	
5/12	L	River bottom	72 hr.	4.04	....	5	
5/16	M	River bottom	72 hr.	4.04	....	6	
5/21	M	Muck	24 hr.	4.04	....	4	
5/31	M	River bottom	120 hr.	4.04	....	7	
5/31	M	River bottom	96 hr.	4.03	....	5	
6/2	M	River bottom	48 hr.	4.03	....	4	
5/26	M	River bottom	48 hr.	4.02	....	4	
5/12	M	River bottom	96 hr.	3.98	....	8	
5/16	M	River bottom	120 hr.	3.96	....	7	
5/12	M	River bottom	120 hr.	3.92	....	7	
5/16	L	River bottom	72 hr.	3.92	....	6	
5/12	M	River bottom	168 hr.	3.88	....	7	
5/21	M	Muck	120 hr.	3.76	....	8	
6/2	M	River bottom	96 hr.	3.72	....	8	
5/19	M	River bottom	72 hr.	3.70	....	7	
5/21	M	Muck	48 hr.	3.69	....	6	
5/21	M	Muck	72 hr.	3.64	....	7	
5/19	L	River bottom	72 hr.	3.52	....	7	
5/28	Ung.		48 hr.	3.40	....	8	Butts
5/21	Ung.		48 hr.	3.37	....	8	Butts
5/28	S.C.		Fresh	3.36	Soup cuts	6	
6/2	Ung.		Fresh	3.26	....	8	Third 5-inch section of stalk

[1] Ung. = ungraded as to size; F. C. = fancy cuts; S. C. = soup cuts; S = small; M = medium; L = large. The last three represent the sizes described by the Agricultural Marketing Administration (1942). [2] Soil type is listed only in the case of samples obtained directly from the growers. [3] Number of hours stored at room temperature before blanching, packing, and freezing. [4] A. I. S. = alcohol-insoluble substances. [5] Predicted grade (F = fancy) is the grade assigned to the commercial product at the factory. [6] All the organoleptic ratings are based solely on tenderness. A sample may be rejected for commercial packing because it is branched but it may still be tender, possibly even more so in some cases, than the unbranched spears. In this column 1 and 2 are high- and low-grade fancy, respectively, 3 and 4 are high- and low-grade extra standard, and 5, 6, 7, 8 represent varying degrees of the reject. It is well to note that border-line samples are probably more accurately judged by the objective test than by the organoleptic. In the organoleptic ratings, it might be better if only three choices had been available to the judges; 1 for fancy, 2 for extra standard, and 3 for off-grade. Such a system would perhaps avoid some of the difficulties that result from too many choices. Actually, the difference between 1 and 2 for fancy as given in the preceding table, and possibly the differences among 1, 2, and 3, in the same table, are debatable. Likewise, the differences among 5, 6, 7, and 8 of the table are debatable.

is perhaps due to the fact that more heads and portions nearest the head went into them, owing to the large amount of spears that were cut too short and that were further cut for this grade.

Based on this work, the following standards of quality for the measurement of toughness of asparagus of the standard five-inch length by means of alcohol-insoluble substances are suggested:

Fancy	4.35% and higher
Extra standard	4.34 to 4.05%
Off grade	4.04% and lower

SUMMARY

An objective method for determining the toughness of frozen asparagus involving the determination of the substances insoluble in 80-per cent ethyl alcohol was found to be successful, as indicated by the coefficient of correlation of these results with the corresponding organoleptic tests. Possible standards of quality based upon this method are suggested.

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VARIATIONS IN CHEMICAL COMPOSITION OF RAW AND CANNED PEAS

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References to the composition of peas found in various texts generally cite the excellent tables prepared by Chatfield and Adams (1940) and by Atwater and Bryant (1906). By reason of the immensity of the scope of these tables it was virtually impossible for the authors to investigate the composition of any one product as completely as might be desired. Earlier work has been published by Bitting (1909), Dubois (1910), Street (1910), and McElroy and Bigelow (1893).

The work reported in this paper is the result of an extensive survey carried out for two purposes: first, to demonstrate the extent of the variation in the composition of raw and canned peas and second, to determine the effect of the canning operation on the composition of peas. The constituents which were determined in this survey are moisture, protein (total nitrogen $\times$ 6.25), ash, ether extract, crude fiber, calcium, and magnesium. In addition, the nitrogen-free extract and the caloric values were computed.

RANGES OF COMPOSITION OF RAW AND CANNED PEAS

Sampling of Raw Peas: Samples of the smooth-skin Alaska variety and samples of four varieties of the sweet or wrinkled-skin raw peas were obtained from one of the major pea-growing sections of Wisconsin during the season of 1941. Each sample was separated according to sieve size into as many fractions as were possible. Duplicate or triplicate portions of each sieve-size fraction were weighed into cans, measured amounts of distilled water were added, and the cans were closed and processed to preserve the sample until such time as the analyses could be made. It is possible that processing the samples in this manner may have resulted in minor changes in the composition of the peas; however, it is believed that any such changes were so small as to be negligible.

Sampling of Canned Peas: As stated, this survey of market samples of canned peas was instituted for the purpose of establishing the range of composition of canned peas as they reach the consumer. With this objective in mind, samples of the 1940 and 1941 packs were obtained from all the major pea-canning districts. Peas of different varieties, of different grades both as to sieve size and quality, and packed in containers of various sizes were analyzed. The samples were further classified according to the process which was used in their preparation. In this connection, brief descriptions of the two canning procedures by which the canned samples included in this study had been prepared, appear to be in order.

The majority of canned peas covered in this study was of the conventional, commercially canned type. In the conventional canning procedure

the peas are washed and sorted in various ways and are then blanched in hot water. The blanched peas are filled into the container, a solution of salt and sugar is added, and cans are closed and heat sterilized. The time and temperature of heat process applied are usually those recommended by The National Canners Association (1942), namely 35 minutes at 115.6°C. (240°F.) or 25 minutes at 118.3°C.(245°F.), but other equivalent processes may sometimes be used.

Also included in this survey were a number of samples canned by a relatively new procedure developed by the American Can Company and generally known as the Blair Process, Blair (1940) and Blair and Ayres (1943). This procedure was developed for the purpose of preserving to a greater extent the natural color and flavor of freshly cooked peas in the canned product. In this procedure a slightly alkaline environment is maintained throughout the process and it is this elevated pH level which is primarily responsible for the greater retention of color and flavor. The first step in the Blair procedure is a pretreatment in a dilute solution of sodium carbonate, after which the peas are drained and washed. The peas are then blanched in a .005 M calcium hydroxide solution. A batch-type blancher is used in order that each small lot of peas may be blanched in a fresh solution. The blanched peas are then washed and filled into the cans; a brine consisting of a very dilute suspension of magnesium hydroxide in a solution of salt and sugar is added; and the cans are closed and processed. Peas packed by this method are processed seven minutes at 126.7°C.(260°F.) and promptly water-cooled. The short high-temperature process is equivalent in sterilizing value to the recommended N.C.A. process and aids materially in the retention of color in the final product.

Analytical Procedure: Analyses were made for the proximate food components, calcium and magnesium. All analyses were made in duplicate, using methods recommended by the Association of Official Agricultural Chemists. Nitrogen-free extract, or carbohydrate, and the caloric value were calculated in the usual manner.

As stated, the raw pea samples had been preserved for analysis by canning in a known amount of water. In preparing these samples for analysis, the net weight of the can contents was determined and the entire contents of the can thoroughly mixed in a Waring Blendor to a smooth purée. In this way it was possible to relate a given weight of the resulting purée to a corresponding weight of the raw peas of the original sample.

In the case of both conventional and Blair-processed peas, as each can was opened the vacuum, headspace, net weight, drained weight, and pH of the brine was determined. In preparing the drained peas for analysis, the peas were thoroughly mixed in a Waring Blendor with the addition of exactly half their weight of water in order to facilitate mixing.

Discussion of Results: The results of the analysis of raw peas are shown (Table 1); the individual analytical values reported are the averages of duplicate determinations, and, in most cases, represent two samples. It is quite apparent that the composition of raw peas as they reach the canner is subject to considerable variation. The moisture content of all varieties shows a definite decrease with increasing sieve size. This increase in total solids parallels a similar increase in the concentration of ash, ether extract,

TABLE 1

Chemical Composition of Raw Peas as Influenced by Variety and Sieve Size

Variety	Sieve size	Number of samples analyzed[1]	Moisture	Ash	Ether extract	Protein	Crude fiber	Carbo-hydrate	Calories per gram	Calcium	Mag-nesium
			pct.	pct.	pct.	pct.	pct.	pct.		pct.	pct.
Alaska	Mixed 1 & 2	2	78.6	0.8	.11	5.5	1.7	13.4	0.8	.029	.034
	3	2	72.7	0.9	.19	7.0	2.0	17.3	1.0	.026	.042
	4	2	71.4	0.9	.21	7.5	2.0	18.1	1.0	.025	.046
Alaska	1	1	81.1	0.7	.07	5.2	1.4	11.7	0.7	.031	.032
	2	3	78.2	0.7	.12	5.7	1.6	13.6	0.8	.030	.036
	3	3	73.0	0.8	.17	6.8	2.0	17.3	1.0	.033	.040
	4	2	71.0	0.9	.20	7.5	2.0	18.6	1.1	.031	.043
Pride	3	2	81.2	0.7	.14	5.4	1.8	10.9	0.7	.027	.031
	4	2	79.2	0.8	.37	5.7	1.9	12.1	0.7	.028	.032
	5	3	78.0	0.8	.41	6.0	2.1	12.7	0.8	.025	.034
	6	2	76.7	0.9	.46	6.5	2.2	13.4	0.8	.026	.033
Alderman	5	2	75.9	0.7	.36	6.8	2.0	14.1	0.9	.026	.037
	6	2	74.1	0.8	.42	7.6	2.1	15.1	0.9	.024	.038
	7	2	73.8	0.8	.44	7.7	2.2	15.1	1.0	.023	.039
Perfection	2	2	83.3	0.6	.16	5.0	1.3	9.7	0.6	.024	.030
	3	2	80.4	0.7	.25	5.9	1.5	11.4	0.7	.029	.033
	4	2	76.4	0.8	.31	7.1	1.7	13.7	0.9	.033	.038
	5	2	74.0	1.0	.36	7.9	1.8	15.0	0.9	.037	.040
	6	2	72.5	0.9	.38	8.5	1.9	15.5	1.0	.039	.040
Profusion	4	2	83.0	0.5	.25	4.9	1.5	9.7	0.6	.024	.033
	5	2	79.2	0.7	.27	6.0	1.7	12.2	0.8	.026	.034
	6	2	76.4	0.8	.31	6.8	1.9	13.8	0.9	.028	.038
	7	2	75.1	0.9	.32	7.2	2.0	14.5	0.9	.029	.039
Minimum			71.0	0.5	.07	4.9	1.3	9.7	0.6	.023	.030
Maximum			83.3	1.0	.46	8.5	2.2	18.6	1.1	.039	.046
Average			76.7	0.8	.27	6.3	1.9	13.9	0.8	.028	.037

[1] Each sample was analyzed in duplicate.

TABLE 2

Chemical Composition of Raw Peas as Influenced by Variety and Sieve Size[1]

Variety	Sieve size	Number of samples analyzed	Total solids[2]	Ash	Ether extract	Protein	Crude fiber	Carbo-hydrate	Calories per gram	Calcium	Mag-nesium
			pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*		*pct.*	*pct.*
Alaska	Mixed 1 & 2	2	21.4	3.7	0.51	25.6	7.9	62.6	3.7	.135	.159
	3	2	27.3	3.3	0.69	25.6	7.3	63.4	3.7	.095	.154
	4	2	28.6	3.1	0.73	26.2	7.0	63.3	3.5	.087	.161
Alaska	1	1	18.9	3.7	0.37	27.4	7.4	61.9	3.7	.164	.169
	2	3	21.8	3.2	0.55	26.1	7.3	62.4	3.7	.137	.165
	3	3	27.0	3.0	0.63	25.2	7.4	64.1	3.7	.126	.148
	4	2	29.0	3.1	0.69	25.8	6.9	64.1	3.8	.107	.148
Pride	3	2	18.8	3.7	0.75	28.7	9.6	58.0	3.7	.144	.165
	4	2	20.8	3.8	1.78	27.4	9.1	58.1	3.4	.135	.154
	5	3	22.0	3.6	1.86	27.2	9.6	57.7	3.6	.114	.155
	6	2	23.3	3.9	1.97	27.9	9.5	57.5	3.4	.112	.142
Alderman	5	2	24.1	2.9	1.49	28.2	8.3	58.5	3.7	.108	.153
	6	2	25.9	3.1	1.62	29.3	8.1	58.3	3.5	.093	.147
	7	2	26.2	3.1	1.68	29.4	8.4	57.6	3.8	.088	.149
Perfection	2	2	16.7	3.6	0.96	29.9	7.8	58.0	3.6	.144	.180
	3	2	19.6	3.6	1.28	30.1	7.7	58.1	3.6	.148	.168
	4	2	23.6	3.4	1.31	30.1	7.2	58.0	3.8	.140	.161
	5	2	26.0	3.8	1.38	30.4	6.9	57.6	3.5	.142	.154
	6	2	27.5	3.3	1.38	30.9	6.9	56.4	3.6	.142	.145
Profusion	4	2	17.0	2.9	1.47	28.8	8.8	56.5	3.5	.141	.194
	5	2	20.8	3.4	1.30	28.8	8.2	58.6	3.8	.125	.163
	6	2	23.6	3.4	1.31	28.8	8.1	58.5	3.8	.119	.161
	7	2	24.9	3.6	1.29	28.9	8.0	58.2	3.6	.116	.156
Minimum			16.7	2.9	0.37	25.2	6.9	56.4	3.4	.087	.142
Maximum			29.0	3.9	1.97	30.9	9.6	64.1	3.8	.164	.194
Average			25.6	3.4	1.17	28.1	8.0	59.4	3.6	.124	.159

[1] Results expressed on the moisture-free basis. [2] Total solids reported on the basis of the fresh raw peas.

protein, crude fiber, and carbohydrate, with a resulting increase in the caloric value. The concentration of calcium and magnesium fails to show any significant trends, although in general the magnesium content increases with increasing sieve size.

Some interesting facts are disclosed when these analytical data are expressed on the moisture-free basis. The results of the analysis of raw peas computed to the dry basis (Table 2) show that, when expressed in this manner, the composition of the raw peas is much more constant. Except for a slight decrease of calcium and magnesium with increasing sieve size, the composition of the different sieve-size fractions of any single variety is quite constant. However, there are marked differences in the average composition of different varieties.

For the results of the survey of commercially canned peas (Table 3) each individual sample was analyzed in duplicate and the average of the several cans taken for analysis is reported. The results refer to the drained

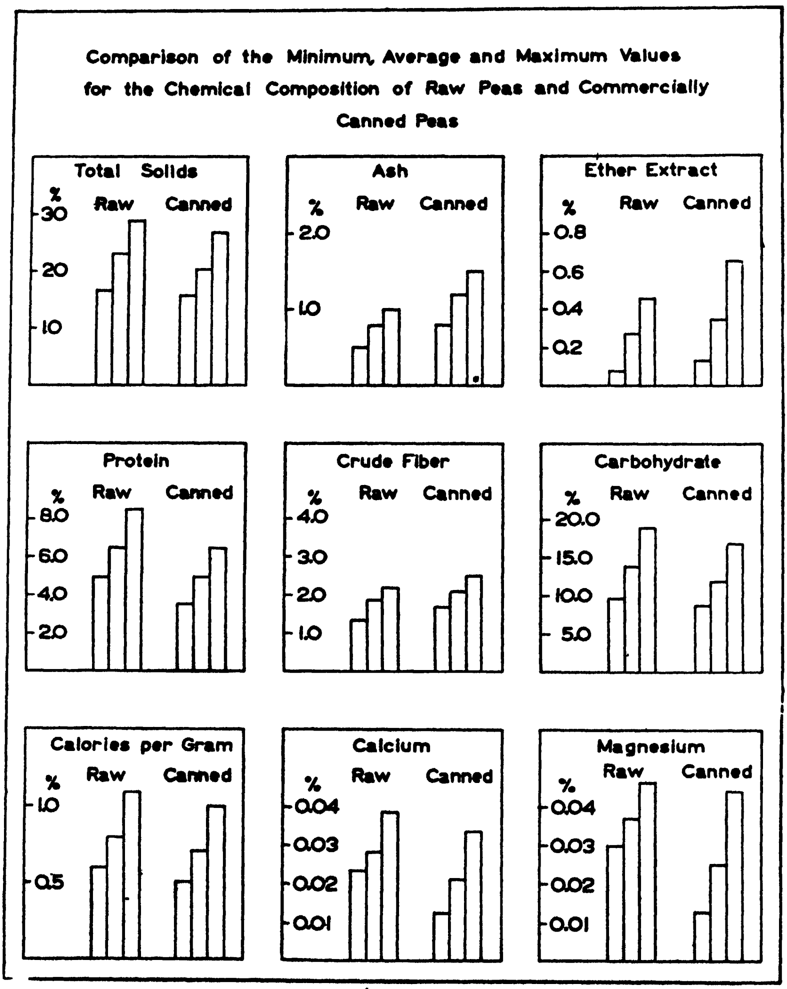

FIG. 1

peas in all cases. Can sizes are designated according to the system of nomenclature used in the canning industry, namely, by the approximate can dimensions. The first digit represents inches, the next two the extra fraction in sixteenths of an inch. The dimension of the can diameter is given first, followed by that of the can height.

It will be readily observed that the variation in composition of the raw canning stock is reflected in a similar variation in the composition of the drained peas. Peas representing the highest quality products contain more moisture, and consequently less protein and carbohydrate, and are therefore lower in caloric value. Peas of medium quality, although not quite so desirable from the standpoint of flavor and texture, have a higher nutritional value, as judged by the proximate composition. Peas packed by the Blair procedure differ from peas of the same grade packed conventionally only in a slight increase in the magnesium content. The magnesium content of the Blair-packed peas is approximately equal to that of raw peas.

TABLE 4

Minimum and Maximum Values for Vacuum, Headspace, Net Weight, and Drained Weight in Commercially Canned Peas

Can size	Number of cans examined	Vacuum (inches of mercury) (inches of mercury)		Headspace (inches)		Net weight (ounces)		Drained weight (ounces)	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
303 x 406	88	0	16	7/32	12/32	16.9	17.7	9.7	11.8
307 x 409	114	0	14	5/32	16/32	20.1	21.7	12.1	15.1
404 x 700	15	0	14	9/32	19/32	50.9	53.4	32.2	34.3
603 x 700	28	0	11	7/32	17/32	110.5	115.0	69.7	80.0

It is to be expected that a comparison of these values for the composition of canned peas with those of raw peas would give a general indication of the effect of the overall canning process on the composition of the peas. In the bar graphs (Fig. 1) the average, maximum, and minimum values for the concentration of each constituent of raw peas and of canned peas are shown. While it is to be understood that no rigid comparison can be made, the data suggest that canned peas are somewhat lower in total solids, protein, and carbohydrate than raw peas. Canned peas contain less calcium than raw peas, and with the exception of peas canned by the Blair procedure, they also contain less magnesium.

All canned samples analyzed were found to fall within the normal limits of variation with respect to vacuum, headspace, and fill as indicated by the data presented (Table 4).

EFFECTS OF CANNING OPERATIONS ON CHEMICAL COMPOSITION

Experimental Procedure: While the foregoing comparison gives a general or overall index as to the influence of the various operations in the canning procedure on the composition of the peas, more exact information would be desirable. With this objective in view, a very closely controlled experimental pack of Alderman sieve size No. 6 peas was made during the 1941 canning season.

TABLE 3

Chemical Composition of Canned Peas as Influenced by Variety, Grade and Sieve Size, Growing Locale, Year of Growth, and Style of Pack

Type of process	Variety	Sieve size and quality	Can size	Year packed	Locale	Number of cans analyzed	Moisture	Ash	Ether extract	Protein	Crude fiber	Carbo-hydrate	Calories per gram	Calcium	Mag-nesium
							pct.	pct.	pct.	pct.	pct.	pct.		pct.	pct.
Conventional	Alaska	Fancy No. 3	307 x 409	1940	Wisconsin	3	76.6	1.1	.25	4.8	2.4	15.1	0.8	.020	.024
Conventional	Alaska	Fancy No. 3	303 x 406	1940	Central Ill., Ind.	2	81.3	0.8	.12	4.1	1.8	11.9	0.7	.015	.021
Conventional	Alaska	Fancy No. 3	307 x 409	1940	Tri-State	3	78.4	1.1	.23	5.0	2.2	13.1	0.7	.019	.021
Conventional	Alaska	Fancy No. 3	307 x 409	1941	Wisconsin	2	81.2	1.1	.20	4.2	1.9	11.4	0.7	.017	.013
Conventional	Alaska	Fancy No. 3	303 x 406	1941	Central Ill., Ind.	3	79.9	0.9	.19	4.6	1.9	12.5	0.7	.015	.019
Conventional	Alaska	Fancy No. 3	307 x 409	1941	New York	2	80.6	1.1	.16	4.2	1.8	12.3	0.7	.012	.013
Conventional	Alaska	Extra Std. No. 3	603 x 700	1940	Wisconsin	2	78.6	1.2	.19	4.5	1.9	14.3	0.8	.022	.024
Conventional	Alaska	Extra Std. No. 3	603 x 700	1941	Wisconsin	3	76.6	1.1	.31	4.9	1.9	15.2	0.8	.023	.021
Conventional	Alaska	Standard No. 4	603 x 700	1940	Wisconsin	2	74.2	1.3	.25	5.8	2.3	16.3	0.9	.029	.032
Conventional	Alaska	Standard No. 4	307 x 409	1940	Central Ill., Ind.	3	75.9	1.0	.23	5.0	2.2	15.6	0.9	.024	.022
Conventional	Alaska	Standard No. 4	307 x 409	1940	Tri-State	3	75.7	1.2	.31	6.0	2.5	14.3	0.8	.021	.026
Conventional	Alaska	Standard No. 4	307 x 409	1941	Wisconsin	2	73.0	1.3	.41	6.4	2.1	17.0	1.0	.024	.028
Conventional	Alaska	Standard No. 4	603 x 700	1941	Wisconsin	3	76.9	1.1	.29	5.2	1.8	14.8	0.8	.022	.021
Conventional	Alaska	Standard No. 4	303 x 406	1941	Central Ill., Ind.	2	74.9	1.0	.34	5.9	2.3	15.6	0.9	.025	.024
Conventional	Alaska	Standard No. 4	307 x 409	1941	New York	2	77.1	0.9	.26	5.2	2.2	14.4	0.8	.016	.018
Blair	Alaska	Fancy No. 4	303 x 406	1941	Wisconsin	4	76.9	1.3	.25	5.2	2.1	14.3	0.8	.020	.042
Blair	Sweet	Fancy No. 3	303 x 406	1940	Minnesota	4	84.1	1.1	.26	4.0	1.8	8.8	0.5	.024	.037
Blair	Sweet	Fancy No. 3	404 x 700	1940	Minnesota	3	84.1	1.0	.26	4.2	1.9	8.6	0.5	.022	.034
Conventional	Sweet	Fancy No. 4	307 x 409	1940	Wisconsin	3	81.2	1.2	.37	4.8	2.1	10.5	0.6	.019	.017
Conventional	Sweet	Fancy No. 4	603 x 700	1940	Wisconsin	3	80.2	1.0	.48	4.8	2.2	11.4	0.7	.024	.021
Conventional	Sweet	Fancy No. 4	307 x 409	1940	Central Ill., Ind.	3	80.1	1.0	.40	5.1	2.2	11.3	0.7	.017	.018
Conventional	Sweet	Fancy No. 4	307 x 409	1940	Washington	3	83.5	1.0	.34	4.1	2.0	9.1	0.6	.017	.017

TABLE 3 *(Concluded)*

Blair	Sweet	Fancy No. 4	303 x 406	1940	Wisconsin	4	82.8	1.2	.36	4.2	2.1	9.6	0.6	.027	.034
Blair	Sweet	Fancy No. 4	303 x 406	1940	Montana	4	83.5	1.2	.49	3.6	2.0	9.2	0.5	.025	.034
Conventional	Sweet	Fancy No. 4	307 x 409	1941	Wisconsin	2	81.2	1.3	.40	5.1	1.9	10.2	0.6	.022	.020
Conventional	Sweet	Fancy No. 4	307 x 409	1941	Central Ill., Ind.	3	80.2	1.2	.47	5.0	2.0	11.3	0.7	.016	.015
Conventional	Sweet	Fancy No. 4	307 x 409	1941	New York	3	78.6	1.4	.41	5.3	2.0	12.2	0.7	.020	.018
Conventional	Sweet	Fancy No. 4	307 x 409	1941	Tri-State	2	79.9	1.2	.43	4.7	2.2	11.7	0.7	.018	.019
Conventional	Sweet	Fancy No. 4	307 x 409	1941	Maine	2	80.4	1.3	.39	4.6	2.2	11.1	0.7	.021	.017
Conventional	Sweet	Fancy No. 4	307 x 409	1941	Washington	2	82.1	1.2	.32	4.1	2.2	10.2	0.6	.019	.019
Blair	Sweet	Fancy Mixed	303 x 406	1940	Wisconsin	3	81.9	1.0	.39	4.6	2.0	10.1	0.6	.031	.035
Blair	Sweet	Fancy Mixed	303 x 406	1940	Wisconsin	3	83.2	1.3	.30	4.3	1.8	9.1	0.6	.029	.040
Blair	Sweet	Fancy Mixed	303 x 406	1941	Wisconsin	4	81.4	1.3	.43	4.7	1.9	10.2	0.6	.024	.044
Blair	Sweet	Fancy Mixed	303 x 406	1941	Wisconsin	4	81.4	1.2	.34	4.6	1.7	10.8	0.6	.019	.043
Blair	Sweet	Fancy Mixed	303 x 406	1941	Minnesota	4	82.8	1.1	.37	4.2	1.9	9.8	0.6	.026	.041
Blair	Sweet	Fancy Mixed	404 x 700	1941	Minnesota	4	81.9	1.2	.39	4.2	1.9	10.4	0.6	.023	.038
Conventional	Sweet	Standard No. 5	307 x 409	1940	Wisconsin	3	80.5	1.2	.40	4.8	2.0	11.1	0.7	.016	.022
Conventional	Sweet	Standard No. 5	603 x 700	1940	Wisconsin	2	81.8	1.0	.38	4.9	2.0	10.1	0.6	.020	.019
Conventional	Sweet	Standard No. 5	307 x 409	1940	Central Ill., Ind.	3	81.5	1.4	.29	4.4	2.0	10.5	0.6	.020	.016
Conventional	Sweet	Standard No. 5	307 x 409	1941	Wisconsin	3	76.7	1.2	.65	5.9	2.4	13.2	0.8	.024	.024
Conventional	Sweet	Standard No. 5	307 x 409	1941	Central Ill., Ind.	2	77.8	0.9	.56	5.8	2.1	12.9	0.8	.026	.019
Conventional	Sweet	Standard No. 5	307 x 409	1941	Tri-State	3	79.6	1.2	.45	5.5	2.0	11.3	0.8	.017	.018
Conventional	Sweet	Standard No. 5	307 x 409	1941	Maine	4	80.2	1.4	.55	4.6	2.2	11.1	0.7	.019	.016
Conventional	Sweet	Standard No. 5	307 x 409	1941	Washington	3	78.4	1.2	.58	5.1	2.5	12.3	0.7	.022	.021
Conventional	Sweet	Standard No. 5	307 x 409	1941	New York	2	75.4	1.5	.50	6.2	2.2	14.3	0.9	.023	.021

TABLE 5

Controlled Comparison of Influence of Conventional and Blair Processes on Composition of Canned Peas

Description of sample	Number of samples analyzed	Moisture	Ash	Ether extract	Protein	Crude fiber	Carbo-hydrate	Calories per gram	Calcium	Mag-nesium
		pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*		*pct.*	*pct.*
Raw peas[1]	3	72.7	1.0	0.46	7.9	2.2	15.8	1.0	.022	.042
Peas after conventional blanch	3	75.0	0.8	0.49	7.4	2.3	14.1	0.9	.021	.037
Drained peas at the end of the conventional process	12	75.5	1.2	0.51	6.7	2.1	14.2	0.8	.020	.031
Peas after the Blair pretreatment	3	74.0	0.9	0.46	7.6	2.2	14.9	0.9	.019	.037
Peas after the Blair pretreatment and blanch	3	75.3	0.7	0.42	7.3	2.3	14.1	0.9	.020	.034
Drained peas at the end of the Blair process	9	75.6	1.3	0.52	6.6	2.1	14.0	0.8	.021	.046

[1] The raw peas used throughout this entire experiment were all of the same lot of Alderman No. 6 sieve peas.

In this controlled pack, peas from the same lot of raw stock were canned by both the conventional and Blair procedures. Samples of the raw peas, as well as samples of the peas taken after each successive operation in the two processes, were taken for analysis. In order to make the analytical results directly comparable, it was necessary to relate all the analyses to some common basis and in this work the analyses have been related to the original raw peas. The weight changes associated with each operation were accurately determined in order that a given weight of peas at any step in the canning procedure could be related to a corresponding weight of raw peas. These weight changes were such that 100 grams of raw peas yielded 92.9 grams of blanched peas and 105.6 grams of drained canned peas. The methods of sample preparation and analysis were identical to those previously described.

Discussion of Results: The results of the analysis of samples in this controlled pack are presented (Table 5); the individual values reported are averages of duplicate determinations on three or more samples. These results confirm the general conclusions made previously. It will be observed that there is a slight but significant decrease in total solids, protein, and

TABLE 6

Minimum and Maximum Values for Vacuum, Headspace, Net Weight, and Drained Weight in Controlled Experimental Pack

Can size	Number of cans examined	Vacuum (inches of mercury)		Headspace (inches)		Net weight (ounces)		Drained weight (ounces)	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
303 x 406	24	0	5	5/32	7/32	17.8	18.2	12.5	12.9

carbohydrate during the blanching operation and that there is a further decrease during the heat sterilization. This latter decrease undoubtedly represents a solution loss in the brine. It will also be noted that there is no significant difference between the effects of the conventional and Blair pea-canning procedures with the exception of a slight increase in the concentration of magnesium in the Blair-process peas. As indicated by the food inspection data (Table 6), the canned samples were closely comparable from the standpoints of vacuum and fill.

SUMMARY

The chemical and proximate analyses of 48 samples of fresh raw peas and of 129 samples of canned peas have been reported. The data show a wide variation in the composition of raw canning stock with a correspondingly wide variation in the composition of the canned peas. Comparison of these data as well as data derived from closely controlled experimental packs shows that there is a slight decrease in the concentration of protein, carbohydrate, calcium, and magnesium during the canning procedure. The effects of the different steps in the canning procedure have been investigated, both for the conventional canning procedure and for a new process known as the Blair process or procedure. Peas canned by the two procedures are similar in composition and differ chiefly in a slightly higher magnesium content in the Blair-process peas.

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EDITORIAL REVIEW

BACTERIOLOGY OF ICE

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The question of survival of microorganisms in ice has long occupied the attention of bacteriologists because of the popular custom of mingling ice with food and drink. Much of the work in this field has been done in the direction of determining the kinds of bacteria which survive or perish during the freezing of water or during the storage period of entrapment in the ice. Obviously most of the experimentation has been directed towards the question of whether or not pathogenic bacteria or other impurities can be found in natural or "artificial" ice when the original water from which the ice was formed contained these bacteria and impurities. The physical nature of ice and the extrusion of bacteria and impurities during freezing has received some study. Since natural ice may be needed to supplement manufactured ice in some sections during the emergency, a review of this subject seems warranted.

NATURE OF ICE

In nature, water is usually frozen into ordinary ice known as ice-I and sometimes into vitreous ice at very low temperatures. There are six other distinct varieties of ice, Bridgman (1912, 1935, 1937). Only ice-I can exist under pressures much less than 2,000 atm. Vitreous ice is formed when small drops of water are chilled quickly to —12°C.(10.4°F.) or lower. This ice, according to Beilby (1921), is very transparent and when examined under the microscope shows no evidence of crystalline structure. Simon (1937) has shown that there are two types of vitreous ice. One is a true glass and the other a supercooled liquid. Hawkes (1929) regards Beilby's ice as merely supercooled water. Herein lies a joint research for physicist and microbiologist for the elucidation of microbial growth in frozen meats and other frozen media. The dictum that some psychrophilic microorganisms will grow at very low temperatures so long as water is available needs to be examined in the light of these studies. Bacteria have been observed to grow in meat at —3°C.(26.6°F.), some at —8°C. (17.6°F.), and a few bacteria and many psychrophilic molds can grow at —12°C.(10.4°F.).

Dorsey (1940) noticed that water at —20°C.(—4°F.) appears as fluid to the eye as water at room temperature. Perhaps a vitreous solid may be supercooled liquid of great viscosity. Dorsey reports that Washburn obtained vitreous ice when the viscosity was increased by the addition of one per cent of sugar.

Some of the ice in quick-frozen animal tissues appears to be vitreous ice.

Ice crystallizes in the hexagonal system. The structure of bulk ice appears to be uniform, but when melting begins at the periphery between the

individual ice crystals, the crystals become more and more separated from one another, and the ice becomes "rotten," Dorsey (1940). Between the crystals is found a material with a lower melting point than the crystal. This material surrounds each crystal enclosing it in a cell—the foam cells of Quincke (1905). When each small unit of water freezes, it rejects impurities. Each minute crystal is surrounded by a layer of water more impure than the original water. As growth takes place in the crystal this layer is pushed out, becoming more impure, but always hugging the crystal until it meets like layers of other cells. Thus each crystal is enclosed in a material having a lower melting point than itself. This is proven by the observation that melting always begins at the boundaries between the crystals. Minute amounts of impurities suffice to produce profound changes in this manner. Bacteria may be extruded from the ice or redistributed in the ice during these changes. Indeed Keith (1913) thought that freezing aqueous solutions of sugar and glycerol caused bacteria to be extruded from the crystals with other matter, while in pure aqueous suspensions of bacteria the cells were mechanically destroyed between growing crystals. He believed that the absence of living bacteria in clear ice and their comparative abundance in "snow" and "bubbly" ice might be explained by this theory. McFarlane (1940) has shown that such factors as rate of crystallization, density and viscosity of medium, gravity, etc., all tend to influence the distribution of suspended and dissolved substances during the freezing process. If conditions are not uniform within the mass, certain areas will be favorable for microbial survival or destruction. When 750-c.c. quantities of water in "malt-quarts" were frozen in still air at —10 or —20°C.(14 or —4°F.) central, vertical, cone-shaped cores of opaque ice and upper horizontal layers were formed. India ink particles were, by freezing, concentrated in the core ice and in and on the horizontal layer. If a solute like one per cent NaCl or one per cent sucrose was present, the distribution pattern of the ink particles was changed in the frozen mass. In 750-c.c. "malt quarts" of cider frozen at —20°C.(—4°F.) microbial forms, soluble solids, and total acids were concentrated in central cone-shaped areas and in surface areas.

In the production of "artificial" ice the water is frozen in a can about the size of a cake of ordinary ice. When distilled water is used, the center core of the cake is not very noticeable; but if ordinary water is used, the minerals and particulate substances will be extruded into a white core. Sometimes this core is not altogether congealed and contains bacteria, suspended matter, and minerals.

In the case of natural ice, Barnes (1925) writes that there is no question from careful conductivity tests that ice, formed on the underside of a thick sheet growing over flowing water, is as pure as it is possible to get anything in nature.

The process of harvesting natural ice must follow sanitary up-to-date methods. The plowing or cutting on the ice field, the practices of the workmen, the floating channels, storage, packing, time in storage, and cleaning for final delivery all must follow bacteriological criteria. When bubble ice or snow ice or foam ice is not shaved from the top surface of the cakes, the sanitary quality of the ice leaves much to be desired, espe-

cially if the ice is not stored for six months before use. Bacteria are usually present in fairly large numbers in this type of surface. The extensive use of electrical refrigeration and "artificial" refrigeration in the home and plant will of course eliminate many of the undesirable features associated with natural ice. On the other hand, artificial ice often contains as many bacteria as were in the water from which it was manufactured. This result may be due to lack of *storage time*—the period in which pathogenic bacteria die off in ice and all nonsporing water bacteria diminish in numbers.

When natural ice is stored, the oldest ice in the icehouse should be "pulled" for current use so that advantage may be taken of the storage-period destruction of bacteria. The time required to produce a sanitary ice by storage is discussed below.

PUBLIC-HEALTH ASPECTS OF ICE

The number of instances of typhoid fever which have been directly traced to ingestion of ice are very few. Prescott and Horwood (1935) state that natural ice obtained from polluted rivers, lakes, and ponds has been harvested and used for years without resulting in definite marked outbreaks of typhoid fever during warm weather. In 1911, Jordan said that "there has never been much danger from the use of impure ice; there need be none." Later Jordan (1938) wrote that when (contaminated) water freezes the great majority of typhoid bacteria that it contains are immediately destroyed. Those that survive die off progressively. The use of natural ice is therefore not so dangerous as the use of the water from which it is formed. "Over 90 per cent both of ordinary water bacteria and of typhoid bacteria die within a few hours (after freezing), and a progressive decline in numbers then takes place; less than one per cent of typhoid bacilli surviving at the end of a week of freezing, according to experiments. Ice stored six months is free from all kinds of bacteria. Outbreaks of typhoid fever have rarely been traced to ice. . . ."

Sedgwick and Winslow (1902), using typhoid bacilli in freezing experiments, showed that 50 per cent reduction took place the first week, 90 per cent the second week, and all at 12 weeks. They considered it a certainty that in nature the destruction would exceed rather than fall short of these figures, because in nature perhaps 90 per cent of the bacteria are extruded during the purifying process of freezing. Park (1920) teaches that freezing does not kill all the typhoid bacilli, but there is a great reduction in their number not only from the act of freezing but also during storage. He states that the danger from the use of ice produced from polluted water is, therefore, much less than from the use of the water itself. "Every week that ice is stored this danger becomes less, so that at the end of four weeks it has become as much purified from typhoid bacilli as if subjected to sand filtration. At the end of four months the danger becomes almost negligible, and at the end of six months quite so. These facts assure us that natural ice which is stored usually for several weeks or months before it is used is practically safe. There is no appreciable increase in the amount of typhoid fever in New York City in the spring when the new crop of Hudson River ice is used. The water from which the ice is taken is moderately infected."

Clark's (1901) chemical and bacteriological studies showed that ice contains less of both bacteria and suspended and dissolved matter than the water from which it is formed. He did not determine the exact time limit for the coliform bacilli and typhoid bacilli to die in ice but stated that they retained their vitality for a number of weeks. He also stated that if there was a considerable depth of water in a polluted pond or river and the ice formed in quiet water, such ice would be entirely satisfactory for domestic use. Thomas (1924) takes exception to the theory stated in textbooks that typhoid bacilli will resist freezing for a considerable length of time. Thomas states that in his experiments the typhoid and paratyphoid bacteria were killed after three weeks of freezing in culture media, water, and feces. Old laboratory cultures of typhoid bacilli were more resistant to freezing than a virulent freshly isolated strain. Reudiger (1911) obtained data from studies with the typhoid bacillus which showed 99.9 per cent destruction of these bacteria during the first eight days of freezing.

Conway (1924) has implicated natural ice in an outbreak of typhoid fever at Elmira, New York. The ice supply was grossly polluted and the superficial layer of ice harvested showed a high total aerobic count and *Escherichia coli* present in .1-c.c. quantities. The ice had been stored for five to six months before use.

Tanner's (1932) conclusions are that "the general consensus of opinion among sanitarians is that ice is not an important epidemiological factor in the spread of typhoid fever."

The question of virus infections from natural ice has received very little study. Extreme cold exerts a preservative effect on viruses and bacteria. Virus-containing tissues can be frozen and stored at —76°C. (—104.8°F.) for months without deterioration of the virus.

Natural ice *grossly polluted* with domestic sewage obviously should not be harvested for edible or potable purposes.

BACTERIA IN ICE

Parker (1941), in discussing the measure of quality in edible substances, pointed out that the kinds of bacteria present, not standard plate counts, determine the type of spoilage which one might encounter and that sanitary control of foods must include qualitative determinations. When qualitative examinations are made there are dispelled those elements of doubt regarding the true significance of practices which might otherwise be controversial. Certain species of bacteria complicate the preservation of food, and it has often been observed that water-borne contaminations are especially troublesome because of the wide temperature range of growth and "hardiness" of these bacteria. The bacteria which can grow well in tap water are often the bacteria encountered in food spoilage, and the literature on food spoilage usually identifies these forms as Pseudomonas, Achromobacter, Serratia, Proteus, and Aerobacter.

Castell and McDermott (1942) observed that the types of bacteria found in water under natural conditions and the types that multiply in water are not necessarily the same. They found the following species to grow actively in tap water at 25° C. (77° F.): *Pseudomonas aeruginosa,*

Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas putrefaciens, Aerobacter aerogenes, Serratia marcescens, and *Achromobacter lipolyticum.*

Members of the genus Bacillus, *Escherichia coli,* Proteus, Micrococcus, and *Alcaligenes viscosus* showed no significant increases over a period of 20 days at 25°C. Castell and McDermott found that these Achromobacter, Pseudomonas, and Alcaligenes species were usually lipophilic, proteolytic, and oxidase positive. Many were psychrophiles. They call attention to the injurious effects of these bacteria on stored meats, fish, butter, etc. Our data show that the bacteria trapped in lake and river ice which grow out on ordinary laboratory culture media are for the most part members of the following genera: Pseudomonas, Spirillum, Chromobacterium, Achromobacter, Serratia, Aerobacter, Proteus, Bacillus, Cellulomonas, Flavobacterium, and Micrococcus. Groups like thermophiles, Streptococci, and *Escherichia coli* are often encountered.

Native water bacteria are not well known because most of them do not grow on laboratory media. Baier (1935) has described these forms, but they apparently do not complicate the preservation of foodstuffs. These bacteria are the spiral forms, the sheathed bacteria like the sulphur and iron bacteria, Sphaerotilus, and some of the nitrifying bacteria.

Other forms which are observed in cake ice are algae, torulae, many of which are chromogenic, rotifers, and the "musty" actinomyces.

During the past 10 years the ice harvested from lakes and rivers of the north central states and examined by our bacteriological staff have shown low counts; i.e., the total numbers of bacteria on nutrient agar plates at 20 and 37°C. (68 and 98.6°F.) have been under 50 for most of the samples. Coliform bacteria were encountered eight times in the 1943 ice harvest on the lakes of the Upper Mississippi River valley near St. Paul. Forty-one cakes of ice were examined and the completed tests for coliform bacteria showed less than 22 per 1,000 c.c. The average "aerobic count" at 37°C. was four and at 20°C. was seven per c.c. The highest count was 40 per c.c. at 20°C. Fifteen samples were sterile or showed less than two bacteria per c.c. at 20°C. Three samples showed 40 bacteria and 23 samples showed three to 10 bacteria per c.c. The bacteria in the ice on the lakes, especially the coliform bacteria, had died out during the period from January 25 to March 11, 1943. None of this low-count ice is used until it has been stored for six weeks or longer.

The temperature of natural ice varies between O and —5°C. (32 and 23°F.), according to Boldyreva (1931). This range of temperature is now considered markedly germicidal. Haines' (1935) data show that the range between —1 and —5°C. (30.2 and 23°F.) is most destructive for *Escherichia coli.* Haines froze suspensions of *Escherichia coli* rapidly at —70°C. (—94°F.) in small tubes, (freezing completed in a few seconds) and stored the tubes at controlled temperatures. The mean count per tube before freezing was 2,000,000 viable cells per tube. After freezing the mean count was 1,360,000 viable cells per tube. The tubes were then stored at —20°C. up to —1°C. for 42 days (Table 1).

It will be observed that death was not most rapid at —20°C. as one would expect. The rate was most rapid in the range of temperatures found in lake and river ice in the United States.

Many observations made in this laboratory show that destruction of bacteria in frozen-egg magma and frozen beef and pork is most rapid when the products are stored at —6.7 to —3.9°C.(20 to 25°F.). However, other factors for conservation, such as humidity, drying, loss of bloom, etc., need to be taken into consideration.

Tanner and Wallace (1931) observed that when *Escherichia coli* are frozen in distilled water and held at —16, —40, and —79°C.(3.2, —40, and —110.2°F.) the cells remain viable for a long time, and no differences in death rates were observed at these temperatures. These ranges are, however, outside the range of natural ice temperatures where the destruction rates are the greatest. Winchester and Murray (1937), utilizing liquid air temperature, observed that *Eberthella typhosa, Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis* survived this temperature for a week. They found that *Eberthella typhosa* withstood this low temperature for 19 months. They concluded that suspensions of bacteria that survive the mechanical effects of freezing are still viable after 19 months at about —83°C.(—117.4°F.) absolute.

TABLE 1
Freezing and Death of Escherichia coli
(R. B. Haines)

Temperature held (°C)	Living cells per tube after days of storage				
	11	18	25	32	42
—20	417,000	260,000	315,000	398,000	367,000
—10	314,000	154,000	134,000	136,000	110,000
— 5	45,800	14,800	5,500	9,000	8,360
— 3	57,300	7,000	2,120	1,820	790
— 2	17,300	....	....	1,000	165
— 1	91,000	2,900	4,600	1,490	1,330

We must conclude from these several investigations that the destructive range of temperature in storage ice lies between —2 and —5°C.(28.4 and 23°F.). All future experiments directed towards determinations of longevity of microorganisms in ice must be rigidly controlled in respect to freezing time, shape of the container during formation of the ice, character of menstrua, storage-period temperatures, and characteristics of the strain of bacteria to be tested.

GERMICIDAL ICE

Several methods have been suggested for treating ice destined to be used in the preservation of fish fillets and other flesh foods. Tarr and Sunderland (1938) reported that a 15-minute dip of fillets in 20 per cent salt brine containing .1 per cent benzoic acid exerted a favorable influence in keeping quality of lightly smoked fish. They pointed out that benzoic acid ice containing .16 per cent of benzoic acid in water freezes at a temperature of 0°C.(32°F.) to form an ice having chemical uniformity incorporated throughout the mass of crystals of an eutectic resembling a true chemical compound. In contrast to such an eutectic ice, the ice formed from solutions of $NaNo_2$ contains the nitrite occluded among

the crystals and there is a tendency for the nitrite to concentrate in the core. These writers had found that nitrite in small amounts (.05 to .2 per cent) in brines inhibited bacterial spoilage in fish fillets to a greater degree than benzoic acid or sodium benzoate. Sodium nitrite was frozen in ice blocks in concentrations of .05, .1, and .5 per cent. The 400-pound blocks of ice formed in two days at —12.2°C.(10°F.). The ice containing .5 per cent nitrite caused absorption of more than 200 p.p.m. by the fish muscle, whereas the lower concentrations did not impart an excessive amount of the nitrite. Fellers and Harvey (1940) found it practical to freeze .5 per cent sodium benzoate in ice cakes under commercial conditions. Since the concentration of benzoate is greatest in the core and lower parts of the ice cake, with the lowest concentrations at the periphery of the cake, the whole cake should be used in crushed form.

Other experimental methods investigated by the author have included the use of chlorine water ice, azochloramide ice, katadyn silver ice, succinyl peroxide ice, calcium or sodium propionate ice, and levulinic acid ice. All of these methods show promise for various purposes.

SUMMARY

In nature water is usually frozen into ice-I and vitreous ice. Ice crystallizes in the hexagonal system. Supercooled water may exist in quickly chilled beef or when drops of water are quickly chilled to —12°C.(10.4°F.) or lower. This may be the state of water when molds and bacteria are observed to grow on meat at —8 to —12°C.(17.6 to 10.4°F.).

Ice crystals grow as water freezes, and a layer of water more impure than the original water surrounds the crystals. Thus each crystal is enclosed in a material having a lower melting point than itself. This is proven when ice melts. The crystals become more and more separated from one another and the ice becomes "rotten." Melting always begins at the boundaries between the crystals. Bacteria, particulate matter, and dissolved substances may be extruded or redistributed in the ice during these changes. Perhaps bacteria are destroyed between growing crystals. Physical factors, such as rate of crystallization, density, viscosity, etc., tend to influence distribution of the trapped substances.

The harvesting of ice must follow "instructions" based upon bacteriological criteria. After ice is stored for several months the nonsporing bacteria are greatly reduced in number. The consensus among sanitarians is that ice is not an important factor in the spread of typhoid fever. The genera of bacteria found in newly harvested lake and river ice are Pseudomonas, Spirillum, Chromobacterium, Achromobacter, Serratia, Aerobacter, Proteus, Bacillus, Cellulomonas, Flavobacterium, and Micrococcus. Bacteria in ice are destroyed more rapidly at the temperatures of natural ice, 0 to —5°C.(32 to 23°F.). At lower temperatures down to that of liquid air, bacteria like the typhoid bacillus remain viable for 19 months or longer.

Several kinds of ice treated with germicides have been used under experimental conditions. Benzoates and nitrites show promise for icing certain foods.

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CARBOHYDRATES OF THE EBENEZER ONION[1]

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The onion is reputed to be the chief food plant in which the reserve material is stored in a bulb. In spite of the fact that they were worshipped in Egypt prior to the Christian era, according to Hill (1937), there appears to be little information in the literature regarding the nature and content of their carbohydrates, which are probably the constituents of greatest potential significance.

Analyses of some varieties have been given by Winton and Winton (1935) and Kihara (1935). Scorodose has been isolated from some varieties but not from others. In some instances fructane with a specific rotation of —41.3° at 18°C.(64.4°F.) has been isolated. The presence of inulin, while suspected, has not been reported.

The data which have been accumulated and which are presented here, deal with the Ebenezer onion. A partial chemical analysis, including some less frequently recorded along with a few more common determinations, on a moisture-free basis, is shown in the following list:

Determinations	Per cent
Total ash	4.54
Total nitrogen	1.98
Total pectic compounds	4.45
Total hemicelluloses	1.88
Total furfural	1.40
Total soluble sugars	64.23
Total reducing sugars	11.34
Total non-reducing sugars	52.89

The soluble sugars may be obtained almost as completely by extracting finely ground tissue with water as by an aqueous alcoholic solution. They are composed of reducing and non-reducing sugars. The ratio of the former to the latter is a variable which is influenced by cultural conditions, length of storage, and conditions of storage. Under optimum conditions the reducing sugars constitute only a small percentage of the total soluble sugars. The nature of these sugars has not been indicated. The non-reducing sugars may be hydrolyzed equally well by mineral acids or by invertase.

In order to obtain some idea as to the nature of the total reducing sugars the proportion of aldose and ketose forms was determined. The aldose sugars were determined by the method of Kline and Acree (1930). This procedure indicated that approximately 69 per cent of the sugars present were of the ketose form. A determination of fructose by the Jackson and Mathews (1932) method accounted for approximately 65 per cent

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of the total hydrolyzed sugars. It would appear, therefore, that the aldose and ketose forms were present in the ratio of about one to two.

Further information as to the nature of the reducing and non-reducing sugars was obtained from their specific rotation before and after inversion, as indicated by Yanovsky (1940). In the majority of instances, depending upon conditions, these were either slightly negative or slightly positive. The average specific rotation of a number of solutions prior to inversion was approximately $[-6°]^{20}_{D}$; after inversion $[-51°]^{20}_{D}$. In these cases the non-reducing sugars were present to the extent of about 32 per cent. This unusually large enhancement in the specific rotation is a strong indication that the bulk of the non-reducing sugars is sucrose.

After the removal of the reducing sugars, which was followed by further purification, and prior to inversion the non-reducing sugars had a specific rotation of about $[+48°]^{20}_{D}$, and after inversion about $[-17°]^{20}_{D}$. These values also strongly support the above indication. The hydrolyzed non-reducing sugars yielded positive results for both Seliwanoff's test and the saccharic acid test, thus indicating the presence of a ketose sugar and either glucose or gulose. These sugars were completely fermented when buffered with a phosphate buffer at pH 6.6 and treated with a purified 20-per cent aqueous suspension of yeast.

Sirups of the total hydrolyzed sugars prepared for crystallization yielded crystals which had a specific rotation of $[-41°]^{20}_{D}$, which is of the order expected for a mixture of d-glucose and d-fructose in the ratio of one to two. They also gave a positive alkali test for fructose, as given by Huntress and Mulliken (1941), and a positive saccharic acid test for glucose. The osazones were characteristic of both sugars. In this case the total sugars were fermented to the extent of about 90 per cent.

The above data would, therefore, indicate that the chief non-reducing sugar in the Ebenezer onion is sucrose. This fraction when hydrolyzed, together with the reducing sugars, may yield a mixture which constitutes nearly 65 per cent of the dry matter nearly all of which may be fermented, and which appears to be approximately two-thirds d-fructose.

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PROTEIN-ASCORBIC ACID COMPLEX IN CARROTS[1]

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Evaluation of the vitamin content of a given food is most satisfactory when estimations by chemical and bioassay procedures are in agreement. Such agreement depends upon several factors. The chemical method must have specificity in the sense that interfering substances may be ruled out and that everything biologically available as the vitamin may be measured. The bioassay must be based upon a control diet adequate in every respect, except for the vitamin in question. The criteria for judging the presence or absence of vitamin deficiency must be delicate enough to permit only a small experimental error.

These ideal conditions are difficult to meet, and because of the time and expense involved in bioassay methods the tendency is to place emphasis on chemical methods. This is especially true where estimation of vitamin potency is part of the control routine, as it should be, in the development of high-grade dehydrated foods. Any information which will contribute to an increased specificity of a chemical method for vitamin estimation, ascorbic acid for example, is distinctly worth while.

The determination of ascorbic acid by means of 2,6-dichlorophenol indophenol, especially the photoelectric adaptation of the method, is one of the most satisfactory chemical methods. The rapidity of the dye reduction, which should be complete in 15 seconds, can be followed by the colorimeter dial readings. In this way ascorbic acid may be differentiated from artifacts causing reduction of the dye at slower rates. In the presence of dehydroascorbic acid, which is known to be biologically available, the method is not quantitative unless precautions are taken to reduce the dehydroascorbic acid. The value of the method may be further enhanced, at least for certain vegetables, by modifications permitting estimation of the ascorbic acid combined with protein.

There have been a number of reports claiming that in the case of certain vegetables, notably cabbage, potatoes, carrots, cauliflower, string beans, squash, parsnips, onions, and peas, cooking appears to increase rather than diminish the ascorbic acid content. McHenry and Graham (1935) have suggested that this is due to an actual increase in free ascorbic acid resulting from the heat decomposition of combined ascorbic acid. These authors obtained similar increases in ascorbic acid values after hydrochloric acid hydrolysis of the vegetable tissues at room temperature. This theory was supported by Ahmad (1935), Guha and Pal (1936), and Levy (1936). Van Eekelen (1935) and Mack (1936) believed that the increase in ascorbic acid value was real and was caused by the heat destruction of "ascorbic

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acid oxidase." Later, Mack and Tressler (1937) reported the production of reducing substances by heating dehydroascorbic acid, and suggested that the apparent increase of free ascorbic acid after heating was due to the formation of such products. Fixsen (1938) reviewed the claims and counterclaims regarding the effect of cooking on the ascorbic acid content of vegetables and, while inclined to accept the "oxidase" theory, suggested that "biological experiments are urgently needed to determine whether the increase represents a real rise, on-balance, of antiscorbutic potency from the point of view of the consumer." Meanwhile, Reedman and McHenry (1938) were working on the biological activity of the material found to be combined with protein. In 1938 they published results which demonstrated that the substance liberated from protein combination by acid hydrolysis was reduced ascorbic acid as shown by chemical, spectrographic, and bioassay examination.

EXPERIMENTAL PROCEDURE

Investigations in this laboratory have repeatedly demonstrated that the residue of carrots, after extraction of free ascorbic acid by metaphosphoric acid, yields an additional reducing substance upon acid hydrolysis. This substance is indistinguishable from ascorbic acid by the photoelectric adaptation of the 2,6-dichlorophenol indophenol method. Upon the suggestion of Dr. A. K. Balls of the Bureau of Agricultural Chemistry and Engineering, who supplied a sample of crystalline chymotrypsin, enzymic digestion of the precipitate obtained from carrots was tested as a means of liberating the reducing substance from protein combination. This procedure offered the possibility of avoiding the production of artifacts by heating or by acid hydrolysis. If artifacts were produced by enzymic digestion, it seemed unlikely that they would be qualitatively and quantitatively the same as those produced by heat or acid hydrolysis. Therefore, a substantial agreement between the value obtained by enzymic digestion and that obtained by acid hydrolysis would constitute additional proof that an increase in reducing power was due to liberated ascorbic acid.

Reedman and McHenry (1938) performed acid hydrolysis with hydrochloric acid at room temperature. In an effort to simplify the process of acid hydrolysis by shortening the time of hydrolysis and by avoiding the necessity of neutralizing the hydrochloric acid so as to obtain the proper pH by buffering of the metaphosphoric acid in the analytical procedure, the following method was used: The precipitate from the metaphosphoric acid extract was suspended in a measured volume of three-per cent metaphosphoric acid and the mixture was boiled for five minutes under a reflux condenser. Boiling for shorter periods gave incomplete hydrolysis, and longer periods led to decreases in reducing power owing to destruction of liberated ascorbic acid. Careful timing of the boiling gave optimum reducing power.

Demonstration of a protein-ascorbic acid complex in carrots by acid hydrolysis and by enzymic digestion was made in the following manner: Thirty grams of fresh carrots were extracted with 300 c.c. of three-per cent metaphosphoric acid in a Waring blender at high speed for 10 minutes. The resulting mixture was divided equally among six 50-c.c. centrifuge tubes. After centrifuging, the supernatant liquid was decanted and filtered

through cotton. Ascorbic acid was determined in the filtrate with 2,6-dichlorophenol indophenol, using Bessey's (1938) photoelectric adaptation of the method. The combined precipitates were transferred to the Waring blender and extracted a second time with 300 c.c. of three-per cent metaphosphoric acid. As before, the mixture was divided equally among six 50-c.c. centrifuge tubes, and analysis was made as described for the first extract. In a typical experiment analysis of the first extract gave a value of 4.2 milligrams of ascorbic acid per 100 grams of fresh carrots. Analysis of the second extract gave a value of zero showing that the first extract of free ascorbic acid was complete.

Having shown that extraction of the free ascorbic acid was complete, the precipitate in one of the centrifuge tubes (equivalent to one-sixth of the total precipitate, or five grams of fresh carrots) was diluted to 50 c.c. with three-per cent metaphosphoric acid and boiled under a reflux condenser for five minutes so as to split any protein-ascorbic acid complex. The solution was then cooled as quickly as possible in running cold water, centrifuged, filtered, and ascorbic acid determined in the filtrate as before. A value of two milligrams per 100 grams of fresh carrots was found. This demonstration of an ascorbic acid value after acid hydrolysis of the precipitate, shown to contain no free ascorbic acid, agrees with many previous observations in this laboratory and indicates the liberation of ascorbic acid from some combination, probably protein.

As a further test for the presence of protein-combined ascorbic acid the following experiment was performed: Chymotrypsin was dissolved in about 125 c.c. of distilled water and the solution was used to wash the precipitate in the remaining five centrifuge tubes (equivalent to five-sixths of the total precipitate or 25 grams of fresh carrots) into a graduated cylinder and was made up to 125 c.c. The pH was adjusted to about 6.5. After thorough mixing, a 25-c.c. sample, representing zero time, was immediately taken, diluted to 50 c.c. with six-per cent metaphosphoric acid and mixed. This procedure gave a sample containing the suspended precipitate, equivalent to five grams of fresh carrots in three-per cent metaphosphoric acid. The sample was centrifuged, filtered, and an analysis made on the filtrate as described before. Similar samples were taken at the end of 15, 30, and 60 minutes and 18 hours. The values obtained for 0, 15, 30, 60 minutes and 18 hours were 0, 1.2, 1.6, 2.3, and 0 milligrams, respectively, of ascorbic acid per 100 grams of fresh carrots. Plotting time up to 60 minutes against concentration of ascorbic acid gave the curve shown (Fig. 1). Plotting time against the logarithm of ascorbic acid concentration gave the curve shown (Fig. 2).

The ascorbic acid value owing to enzyme digestion of the protein was close to maximum at the end of 60 minutes (Fig. 1). This value agrees reasonably well with the value obtained after acid hydrolysis. This close agreement suggests that the same substance has been liberated in both cases and that it is ascorbic acid. Moreover, the finding of a zero value after allowing the chymotrypsin to act for 18 hours is consistent with the probable oxidation of liberated ascorbic acid at the pH of approximately 6.5. The instantaneous reduction of the 2,6-dichlorophenol indophenol by the liberated substance, as observed in the photoelectric colorimeter, shows that

the product is not an artifact with less energetic reducing powers than ascorbic acid. Finally, the reaction taking place in the presence of chymotrypsin is a time reaction of the first order and as such suggests the liberation of something nonprotein from a protein complex, because strict proteolysis is not usually a first-order process.

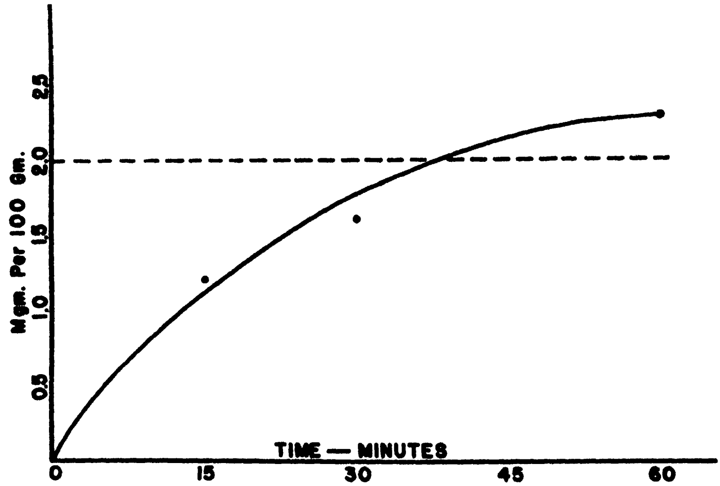

FIG. 1. Milligrams of ascorbic acid per 100 grams of fresh carrots liberated from protein combination by (a) acid hydrolysis after boiling five minutes with acid (dotted line), (b) digestion of protein by chymotrypsin (solid line).

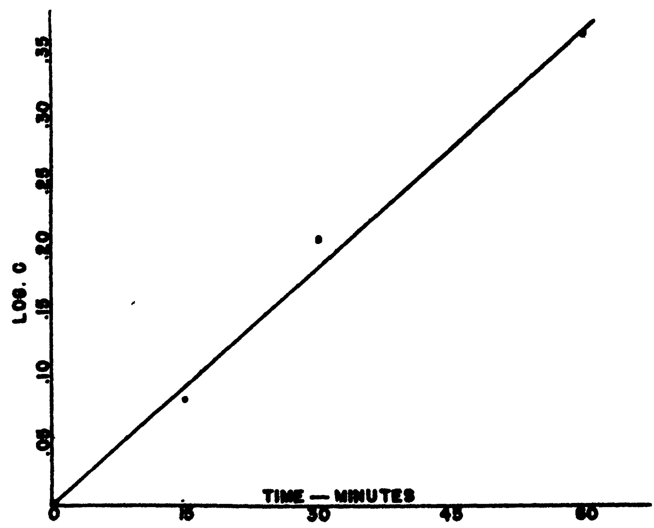

FIG. 2. Data of chymotryptic digestion plotted on logarithmic scale.

CONCLUSIONS

The results obtained with carrots agree with the claims of others that certain vegetables contain protein-combined ascorbic acid. Investigations on the general occurrence of such combined ascorbic acid should be extended. Since ascorbic acid in this form is biologically available, it

must be taken into consideration in determining the value of different foods and in comparing the bioassay and chemical methods. The degree of protection which such a combination may afford ascorbic acid during dehydration and processing of food is worthy of investigation.

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ALCOHOL-GLYCEROL RATIO OF CALIFORNIA WINES

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The early enologists considered the alcohol-glycerol ratio to be important in the detection of sophistication in wines. Later, Kayser (1905) and other investigators of fermentation chemistry found the ratio of alcohol to glycerol to be affected by type of yeast, sulfur-dioxide content, pH, temperature, concentration of the different types of sugars and acids, and other factors. The ratios reported in the literature in wines were, therefore, highly variable and led some to consider that the alcohol-glycerol ratio could be of little value in the detection of watering, fortification, and other fraudulent practices of the table-wine industry.

Since we know, however, that glycerol is derived from the sugars during fermentation, the amounts found in wines are really of interest. The fermentation procedure for dry table wines in most wineries is fairly standardized, and a nearly constant alcohol-glycerol ratio may be expected in these wines. The fortification time of the various types of dessert wines, however, is extremely variable, and a considerable range in the apparent alcohol-glycerol ratio should be found in commercial dessert wines. In dry wines, furthermore, glycerol constitutes the largest portion of the extract and is undoubtedly of organoleptic importance.

As Seifert and Reisch (1904) have definitely shown, less glycerol is formed per gram of alcohol produced when the sugar content is increased. Higher alcohol-glycerol ratios would, therefore, be expected from the fermentation of the high-sugar California musts as compared with the comparatively low-sugar European musts, but other factors complicate the relationship. Joslyn (1940), for instance, reports that glycerol formation in general varies inversely with the activity of the yeast. The rapid California fermentations might therefore be expected to produce less glycerol. Furthermore, tartaric acid is generally found to increase glycerol production, and since the California musts used for dessert-wine production are very low in tartaric acid, the relative amounts of glycerol produced should be lower. The high sulfur-dioxide content of California musts intended for table wines would be expected to increase glycerol formation. Sulfur dioxide is used by some California wineries producing dessert wines, but others do not utilize it. The ratios found in these wines will vary slightly because of this.

According to von der Heide (1922), Gunther found 90 per cent of 4,423 German wines tested between 1901 and 1911 to have alcohol-glycerol ratios of nine to 17. Less than four per cent had ratios above 20 or below eight. Considering that some sweet *auslese* wines were probably included in the analyses and that analytical difficulties are inherent in determining the glycerol content of sweet wines, one may safely say that ratios of eight to 20 are the maximum range to be expected in German wines.

Joslyn (1940) indicates that ratios of seven to 17 may be expected in wines. Similar references to the French enological literature could be given. According to French law the maximum for table wines should not exceed 17.

Bigelow (1900) and Wiley (1903) have published fairly extensive alcohol and glycerol analyses of California wines. Bigelow analyzed commercial wines together with some of Hilgard's experimental wines from the California Agricultural Experiment Station; Wiley analyzed commercial wines exhibited at the Paris exposition of 1900; their results are summarized (Table 1).

The ratios found by Bigelow in table wines, being much higher than those reported in the European literature, led him to suggest that a higher maximum would be necessary for California table wines, but the variability of his experimental material was great. He used many wines produced for grape-variety testing by Hilgard—varieties of low sugar or anomalous composition and of no commercial importance being included—and the extreme variability in his results is probably explained by this fact. Wiley's ratios for red and white commercial table wines, however, fall fairly well within the European limits, and his data are much less variable than Bigelow's.

In fortified wines high ratios and widely varying results were found by both Bigelow and Wiley and are to be expected. Before prohibition the fortification practices in California wineries were apparently even less standardized than at present.

The wines selected for the present study were those exhibited for judging at the 1939 Golden Gate International Exposition. Duplicate unopened bottles submitted for the judging were secured from the judges for analysis.

The glycerol was determined by the periodic acid method of Amerine and Dietrich (1943). Bigelow and Wiley's glycerol analyses (Table 1) were obtained by the usual calcium-oxide clarification procedure and direct weighing. Since, however, this method is indefinite and erratic for sweet wines, some of their results may be in error. Alcohol was determined by hydrometer on the distillate.

CALCULATION OF THE RATIO

With unfortified wines one encounters no difficulties in calculating the ratio. The glycerol and alcohol are expressed as grams per 100 ml., and the division is made. Wines not completely fermented—Sauternes, for example—are calculated in the same fashion except that the ratio should be reported as the apparent alcohol-glycerol ratio. The maximum glycerol content has not, of course, been attained since the wine still contains unfermented sugar. Since sulfur dioxide is known to exert a favorable influence upon glycerol formation, one would expect the alcohol-glycerol ratio to be rather low in this type of wine—that is, more glycerol should be formed per gram of alcohol produced. Some producers of the sweeter Sauternes types, however, add some alcohol or some fortified wine during production, and this would increase the ratio.

With fortified wines not all the original sugar has fermented, and the alcohol added during the fermentation introduces an error in the ratio

TABLE 1

Alcohol-Glycerol Ratio of Pre-Prohibition California Wine

Wine type	Analyst	Number of samples	Alcohol	Glycerol	Alcohol: glycerol			Extract	Alcohol: glycerol[1]
					Maximum	Average	Minimum		
			gm./ 100 ml.	*gm./ 100 ml.*					
White:									
Dry[2]	Bigelow	52	9.93	.647	29.7	15.3	7.2	......	......
Sauternes[3]	Bigelow	38	10.30	.571	56.8	18.0	12.1	2.58	......
Dry	Wiley	13	9.88	.714	17.5	13.8	10.9	......	......
Fortified, dry[4]	Bigelow	7	15.70	.524	49.4	30.0	20.3	5.82	13.8
Fortified, sweet[5]	Bigelow	6	14.20	.843	46.4	16.9	11.1	17.69	1.68
Fortified	Wiley	7	16.26	.281	348.5	57.3	26.9	14.59	9.54
Red:									
Claret	Bigelow	38	10.00	.542	29.8	18.5	11.5	......	......
Burgundy, etc.[6]	Bigelow	42	9.83	.554	22.5	17.8	13.9	......	......
Dry	Wiley	16	9.92	.666	17.0	14.9	12.3	......	......
Fortified	Bigelow	13	15.32	.550	87.1	27.9	18.5	12.05	7.42
Fortified	Wiley	6	16.50	.612	56.4	27.0	22.3	16.01	3.14

[1] Calculated by special formula (3) for fortified wines. [2] Includes Rhine, Burgundy, southern French, and other types. [3] Primarily dry wines from Sauterne-type grapes. [4] Sherry. [5] Angelica and muscatel. [6] Includes Jura, Italian, southern French, and other types.

and a dilution factor. If one knew the original sugar content of the grapes, a close approximate calculation of the alcohol derived solely from fermentation could be made. As a general average, an original Balling (soluble solids content) of 25° can be assumed for California wines. The alcohol derived from fermentation expressed in milliliters per 100 ml. of unfortified wine is then given by the equation [1]

$$F = \frac{1375 - 13.75\,A - 55\,E'}{100 - A - 0.55\,E'} \qquad (1)$$

where A is the total alcohol content of the fortified wine and E′ is the extract of the fortified wine. F gives, in volume per cent, the alcohol produced by fermentation in the wine before fortification.

The dilution effect of the fortification on the percentage glycerol can also be calculated. G, the glycerol value corrected for the dilution effect of fortification, is equal to [2]

$$\frac{G'\,(100 - F)}{(100 - A)} \qquad (2)$$

The complete equation for the calculated alcohol-glycerol ratio of dessert wines is then [3]

$$\frac{\text{alcohol}}{\text{glycerol}} = \frac{(100 - A)F^{*}}{(100 - F)G'} \qquad (3)$$

In this equation F has the value as calculated in equation (1) as milliliters per 100 ml. F* has the corresponding value of F expressed in grams per 100 ml. G′ is expressed in grams of glycerol per 100 ml. of fortified wine and A is the total alcohol content in milliliters per 100 ml.

Obviously, a direct calculation of the alcohol-glycerol ratio in fortified wines would yield misleadingly high results. Bigelow (1900), for instance, noted the high ratio (actually he calculated the glycerol-alcohol ratio, which was low) of California dessert wines. When his results and Wiley's are recalculated on the basis of the formula just given, they are more nearly concordant (Table 1). As one can readily see, this high apparent

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[1] $F = 0.55\left[25 - E' \times \frac{100}{100 - A + F'}\right]$ where E′ represents the grams of extract per 100 ml. for fortified wine and F′ is the ml. of alcohol derived from fermentation per 100 ml. of fortified wine. It can also be shown that

$$E = E' \times \frac{100}{100 - A + F'} \qquad \text{and } F = F' \times \frac{100}{100 - A + F'} \qquad \text{or } F' = \frac{(100 - A)F}{(100 - F)}$$

[2] $G = G' \times \frac{100}{100 - A + F'}$ and as above $\frac{(100 - A)F}{100\,F}$ is substituted for F′.

[3] $$\frac{F}{G} = \frac{F}{\dfrac{G'(100 - F)}{(100 - A)}}$$

TABLE 2

Alcohol-Glycerol Ratio of Post-Prohibition California Wines

Wine type	Number of samples	Alcohol	Glycerol	Alcohol: glycerol			Extract	Alcohol: glycerol[1]
				Maximum	Average	Minimum		
		gm./ 100 ml.	*gm./ 100 ml.*				*gm./ 100 ml.*	
Nonfortified:								
Chablis	12	9.75	0.90	12.0	10.7	9.8	2.2	......
Hoch and Moselle	12	10.0	0.93	12.3	10.6	9.0	2.1	......
Riesling	9	9.3	0.90	11.5	10.3	7.9	2.2	......
Sauternes, dry	21	9.8	0.98	12.9	10.0	8.5	2.7	......
Sauternes, sweet	10	10.15	1.11	11.9	9.2	5.6	4.8	......
Sauternes, Chateau	4	9.4	0.93	11.8	10.1	9.2	5.1	......
Barbera	8	9.75	1.03	10.3	9.3	8.1	2.9	......
Burgundy	19	10.0	1.14	10.2	8.8	5.0	2.8	......
Cabernet	7	9.8	1.01	11.1	9.5	8.9	2.8	......
Claret	3	9.75	1.05	9.9	9.2	8.5	2.8	......
Zinfandel	19	10.3	1.02	11.0	9.7	7.4	2.8	......
Fortified:								
Angelica	15	16.3	0.66	32.9	25.4	13.2	13.5	4.85
Muscatel	16	15.8	0.57	42.1	28.5	19.7	13.9	5.46
Port	25	15.9	0.53	46.9	29.9	13.3	12.4	7.27
Sherry, dry	19	16.0	0.96	18.7	16.4	13.6	3.3	8.75
Sherry, sweet	29	15.9	1.02	19.5	15.6	9.7	5.2	7.36
Tokay	7	15.9	0.64	28.7	24.6	20.5	11.8	6.13
White port	6	16.0	0.50	43.7	35.7	19.8	12.9	7.12

[1] Calculated by special formula (3) for fortified wines.

ratio in fortified wines is due primarily to fortification and not to dilution with water. The excessive use of water in crushing grapes is not uncommon, however, in California. This type of fraud may sometimes be detected by ascertaining the acid and extract ratios, together with the nature of the acids present.

RESULTS

The results obtained on the 1939 wines show that in nonfortified wines the ratios found are well within the range reported in the European literature (Table 2). Only one sample had a ratio as low as five, and the average was remarkably constant. The maximum ratio never exceeded 13.

In the fortified wines results were somewhat more variable, none, however, proving so divergent as those reported by Bigelow and Wiley. When recalculated according to the proposed formula, the ratios were more nearly constant and fell slightly below the expected range. Whether this lower ratio is due to higher glycerol production in dessert-wine fermentation or to an incorrectly assumed original Balling is not known.

SUMMARY

The alcohol-glycerol ratio of commercial post-repeal California table wines is less variable than that reported in the pre-prohibition literature. It is well within the range of normal wines, and the averages for the various types of table wines are remarkably constant. The ratios found in present-day dessert wines are reasonably constant and, when recalculated by the proposed formula, are slightly lower than those of the table wines.

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MORPHOLOGICAL AND PHYSICOCHEMICAL DIFFERENTIATION IN VARIOUS LAYERS OF AVIAN ALBUMEN

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Albumen of the avian egg is a secretion of the oviduct. In its consistency it represents a semiliquid, jelly-like substance with a slightly yellowish tint, enmeshed in a thin network of fibrous matter. The albumen of the fresh egg is an almost clear substance, but in a slightly older egg it becomes cloudy or opalescent in its appearance.

It has been known since Coste (1847) that the egg albumen consists of four distinct layers, but the biological significance of these layers is still unknown. In order to determine this, it is necessary to have a more complete picture of the morphological structure as well as of the physical and chemical properties of each layer. This information should be of importance especially in ascertaining the physiological reasons for the morphological structure.

MORPHOLOGICAL STRUCTURE

The innermost layer of dense albumen encircles tightly the vitelline membrane of the yolk. This layer is often referred to as the "membrane chalaziferae." It consists of a thickened, viscous, mucus-like, semifluid substance and at the opposite poles of the yolk is drawn out into threads which are known as chalazas.

The chalazas in chicken eggs were found by the author to differ in length; the longest one was consistently that which is attached to the yolk at the sharp end of the egg. It was also observed by Almquist (1936) that the chalaza at the blunt end of the egg invariably has a clockwise twist, while that at the sharp end has a counterclockwise twist. The different directions of twist may be explained by the rotation of the yolk in a counterclockwise direction as it moves down the oviduct.

The middle layer of thin or fluid albumen constitutes the second layer, and it enwraps the innermost dense albumen.

This middle layer is next encircled by the albuminous sac or middle layer of thick albumen which is of decidedly denser consistency than the preceding layer, though not as dense as the innermost layer. It is jelly-like, muculent, and adhesive in its consistency and constitutes the largest bulk of all the layers.

It is interesting to note that the dense albuminous sac is attached to the inner or egg membrane at both the blunt and sharp ends of the egg. From the measurement of many chicken eggs it was found that the area of attachment, which is also known as "ligamentum albuminus" (Bartelmez, 1912), was consistently greater at the blunt end. This surface area was, on the average, about 10.2 sq. cm., while that of the sharp end was only about 2.54 sq. cm.

The difference in surface area of attachment may well account for the presence of the air sac almost invariably at the blunt end of the egg. As

the air sac forms following laying, a greater pull of inner membrane would be exerted at the blunt end with the contraction of the albumen on cooling.

The outermost layer of albumen has a clear, thin, liquid-like, almost watery consistency, otherwise possessing the same general characteristics of albumen.

METHODS OF SEPARATION

A "pipette" method for the separation of the layers of albumen was suggested and then briefly described by the author (Romanoff, 1929; and Romanoff and Sullivan, 1937). However, the "screen" method of separating the albumen (Holst and Almquist, 1931), has continued to prevail.

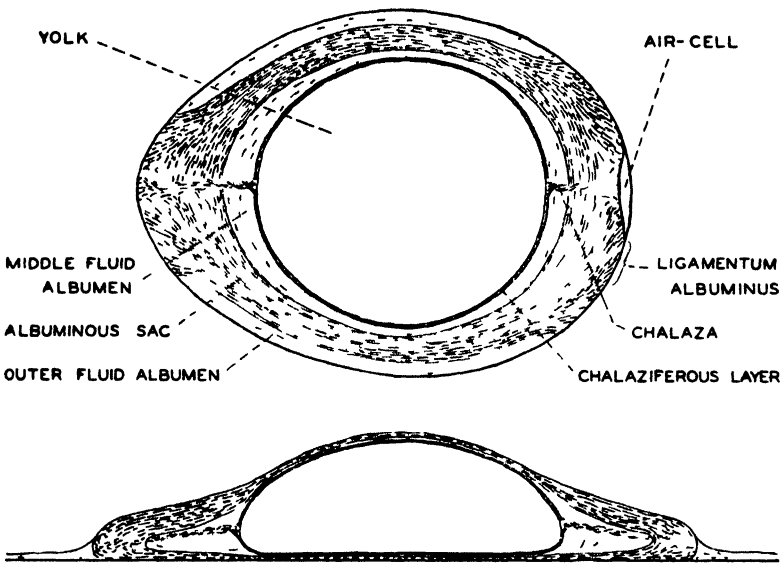

FIG. 1. Diagrammatic cross sections of intact and opened egg showing the relationship between different layers of albumen of chicken eggs. [For a detailed study of egg yolk the reader is referred to the author's paper on the "Growth of Avian Ovum," Anat. Rec. (1943), Vol. 85, pp. 261-267].

The latter method, owing to the rupture of the albuminous sac, results in the combination of the middle-thin and outer-fluid layers, so that the measurements are made of "thick" and combined "thin" portions. The method used to obtain the present data is as follows:

The contents of the egg are gently dropped into a large Petri dish and the layers of albumen removed successively by means of a pipette. The outer thin layer is removed first with a pipette having a one-mm. bore at the point. Then an incision is made in the dense layer with scissors; the middle-thin layer is allowed to run out and is removed by the same size of pipette. The middle-dense layer requires the use of a pipette with a larger bore (two mm.) at the point. The innermost chalaziferous layer and chalazas must be removed with forceps.

PROPORTIONAL AMOUNTS

These four layers of albumen constitute the white of the bird's egg which occupies about two-thirds of the space within the egg. The pro-

portional amounts of each layer in chicken eggs were found by Romanoff and Sullivan (1937) to be on an average as follows: the innermost chalaziferous layer 2.69 per cent, the middle-thin layer 16.84 per cent, the middle-thick layer 57.29 per cent, and the outer-fluid albumen layer 23.18 per cent. The percentages were found to be very consistent within the species, irrespective of the breeds of fowl, by Hall (1939).

On the other hand, the proportional amounts of the four layers of albumen vary considerably in these different species of birds: Bobwhite quail *(Colinus virginianus)*, Ring-necked pheasant *(Phasianus torquatus)*, guinea fowl *(Numida meleagris)*, Leghorn chicken *(Gallus domesticus)*, White Holland turkey *(Meleagris gallopavo)*, mallard duck *(Anas boschas)*, Peking duck *(Anas domesticus)*, and Muscovy duck *(Cairina moschata)*. However, certain generalizations may be readily agreed upon. It is evident (Table 1) that the innermost layer of dense albumen has consistently the smallest and the middle-thick layer has the largest percentage weight of all layers. The outermost layer is proportionately smaller in weight to the adjacent middle-thick layer of albumen. The only exception to this is in the eggs of the water fowls, mallard, Muscovy, and Peking ducks, in which the proportional weight of the outer thin layer is nearly equal to that of the middle-thick layer.

TABLE 1

Proportional Amounts of Four Layers of Egg Albumen in Various Species of Birds[1]

Layers of albumen	Land fowls					Water fowls		
	Bob-white quail	Ring-necked pheasant	Guinea fowl	Leghorn chicken	White Holland turkey	Mallard duck	Peking duck	Muscovy duck
Weight (grams):								
Outer	2.23	6.06	4.30	7.85	7.43	12.08	17.95	16.27
Middle-thick	1.95	8.02	11.83	19.18	25.27	12.74	18.90	16.86
Middle-thin	0.30	2.34	3.55	5.64	10.70	5.13	2.35	3.53
Inner	0.13	0.20	0.20	0.90	0.79	0.33	1.25	0.24
Total	4.61	16.62	19.88	33.57	44.19	30.28	40.45	36.90
Proportion (per cent):								
Outer	48.37	36.48	21.63	23.38	16.81	39.90	44.38	44.09
Middle-thick	42.30	48.26	59.51	57.14	57.18	42.07	46.72	45.69
Middle-thin	6.51	14.08	17.86	16.80	24.21	16.94	5.81	9.57
Inner	2.82	1.20	1.00	2.68	1.80	1.04	3.09	0.65
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Egg weight (grams)	8.95	29.80	39.50	60.10	81.67	58.69	74.70	75.55

[1] Based on the averages of from 5 to 30 eggs of each species.

PHYSICOCHEMICAL PROPERTIES

The data presented clearly indicate that in all species the percentage of dry matter in the albumen increases consistently from the outer to the inner layer (Table 2). This agrees with the general conclusions previously made by Romanoff (1929) and then presented with further data (Romanoff and Sullivan, 1937). Work by Moran (1937) has also confirmed this increase in the percentage of solids.

TABLE 2

Physicochemical Properties of Various Layers of Egg Albumen

	Number of analyses	Layers of egg albumen			
		Outer	Middle-thick	Middle-thin	Inner
Dry matter		pct.	pct.	pct.	pct.
Chicken	57	10.70	12.85	13.72	15.82
Pheasant	12	10.97	12.04	13.66	14.66
Quail	10	11.88	12.81	14.20	15.10
Turkey	13	14.78	17.15	18.15	19.29
Peking duck	8	12.64	12.94	13.28	14.95
Runner duck	10	12.57	13.70	15.23	16.40
Total nitrogen (on dry basis) Chicken	3	13.40	13.62	13.78	13.91
Total ash (of dry matter) Chicken	5	2.32	2.52	2.87	1.86
Calcium (CaO) of ash Chicken	5	6.49	7.58	9.02	8.98
Density		sp. gr.	sp. gr.	sp. gr.	sp. gr.
Chicken	5	1.0315	1.0346	1.0369	1.0469
Pheasant	6	1.0366	1.0365	1.0381	....
Quail	5	1.0377	1.0401	....	....
Turkey	8	1.0349	1.0394	1.0428	....
Peking duck	6	1.0419	1.0402	1.0417	....
Muscovy duck	4	1.0413	1.0397	....	....
Refractive index		$n_{(D)}$	$n_{(D)}$	$n_{(D)}$	$n_{(D)}$
Chicken	80	1.3509	1.3540	1.3578	1.3613
Pheasant	6	1.3528	1.3567	1.3575	1.3685
Quail	5	1.3571	1.3581	1.3590	1.3603
Turkey	....	1.3535	1.3561	1.3594	1.3628
Peking duck	4	1.3542	1.3557	1.3569	1.3612
Runner duck	5	1.3564	1.3575	1.3597	1.3612
Hydrogen-ion concentration		pH	pH	pH	pH
Chicken	3	7.98	7.92	......	......
Pheasant	7	8.38	8.13	8.57	......
Quail	....	9.42	9.32	......	......
Turkey	5	8.25	7.81	7.92	7.76
Duck	4	8.69	8.53	8.54	8.65
Audio-frequency conductivity		(MHO — $cm.^{-1}$) $\times 10^{-3}$			
Chicken	6	8.82	9.63	8.84	......
Pheasant	4	7.93	8.49	8.33	......
High-frequency conductivity		Relative (galv. scale read.)			
Chicken (fertile)	28	14.10	15.90	11.15	......
Chicken (infertile)	28	16.15	17.03	12.35	......

The data on refractive index, audio-frequency and high-frequency conductivity are in good agreement with the previously published ones by Romanoff and Sullivan (1937), Romanoff and Grover (1936), and Romanoff and Frank (1941).

The values for the percentage of total nitrogen as determined for chicken eggs on a dry basis, also show a similar rise from the outer albumen layer toward the yolk. This was expected to correspond with the increase in dry matter of the albumen. Similar changes were observed in the density of albumen with increasing values from the outer to the inner layer in all species studied. The refractive index of the various layers of albumen for the same species shows a similar increase from the outer layer toward the yolk.

There is apparently little variation or definite change in hydrogen-ion concentration from layer to layer of albumen in any species. The middle-dense layer of albumen in both chicken and pheasant shows a higher conductivity (audio and high frequency) than the other layers. The heat coagulation points for the outer, middle-thick, and middle-thin layers of albumen are 61.5, 61, and 60.3°C.(142.7, 141.8, and 140.6°F.), respectively.

DISCUSSION

The proportional amounts of albumen in the four layers are known to vary with the individual hens within the species. Holst and Almquist (1931) showed that the percentage of thick white varies with the individual hen; but tends to be independent of variations in egg size and in total amount of white (Knox and Godfrey, 1934). The refractive index of the albumen is characteristic of individual birds, and the relation between the different layers does not change (Romanoff and Sullivan, 1937).

The percentage volumes of layers vary with the age of the egg. The middle-thick layer decreases sharply as the outer-thin layer rapidly increases in percentage volume (Moran, 1937). Our observations show that this disintegration of the sac of thick albumen is the most marked change in the egg during storage.

Other less obvious changes also occur. In none of the experiments has it been found possible to preserve the physicochemical condition of the egg as it exists when it is laid. So it is clear that in this sense the new-laid egg is not an equilibrium system. Rather, it is chemically and physically unstable. This instability varies markedly with the temperature—the higher the temperature the more rapid the deterioration (Romanoff, 1940). At the incubation temperature of 37.5°C.(99.5°F.) the differentiation of the albumen into four distinct layers, as indicated by variation in refractive index by Romanoff and Sullivan (1937), may practically disappear within two or three days. This change does not occur at 20°C.(68°F.) until after five or seven days, while at 0°C.(32°F.) it would probably be possible to hold the egg 10 to 12 days without marked deterioration in the layers.

Besides the variation in the layers of albumen that may occur with different hens, aging, and holding temperatures, there is also known to be variation within the breeding season. A study of the refractive index of the albumen layers by Romanoff and Sullivan (1937) indicates that the refractive index of all layers is highest just at the beginning of the natural breeding season and lowest at the close. It was found that differences in the refractive index among the eggs of individual hens may have some biological significance. Low fertility in eggs is apparently associated with a low refractive index of albumen, although hatchability of the fertile eggs shows no such correlation.

SUMMARY

The albumen of the new-laid avian egg is differentiated into four layers with distinct chemical and physical properties. In general, the middle-thick layer has the greatest percentage weight and the innermost-dense layer the smallest, while the percentage weight of the outermost exceeds that of the middle-thin layer.

In eight species studied the percentage of dry matter and total nitrogen, density, and refractive index consistently increase in value from the outermost to the innermost layer.

There is little variation in the hydrogen-ion concentration from layer to layer of albumen, but the middle-dense layer in chicken and pheasant shows a higher audio- and high-frequency conductivity than the outer and especially middle-thin layers. Heat coagulation points in chicken eggs are 61.5, 61, and 60.3°C. for the outer, middle-thick, and middle-thin layers, respectively.

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MACARONI COOKING VALUE OF SOME NORTH DAKOTA DURUM WHEAT SAMPLES [1]

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Methods of determining "cooking value" of macaroni have been described by Borasio (1935), Binnington, Johannson, and Geddes (1939), and Harris and Knowles (1939, 1940). Standardized procedures were developed by these authors and applied to the detection of the quality differences among durum wheats and locations of growth. The principal factors included in the determination of cooking quality have been considered to be increase in volume of the macaroni, degree of disintegration upon cooking, and tenderness of the cooked product. Increase in volume was originally determined by the actual gain in volume of the macaroni following cooking under strictly specified conditions. Later, when it was found that a high positive correlation existed between increase in volume and gain in weight, the latter value was used as a measure of the former. Degree of disintegration is determined by the weight of residue found following evaporation of the filtrate after removal of the cooked macaroni on a Büchner funnel. The tenderness of the cooked sample is calculated from curves traced by a tenderness tester, using the apparatus and technique described by Binnington, Johannson, and Geddes (1939) and employed by Harris and Knowles (1940) in determining the tenderness score of 17 samples of North Dakota durum wheat produced in 1938.

EXPERIMENTAL PROCEDURE

The present paper is concerned with the application of these techniques to the evaluation of the cooking quality of a number of durum wheat samples and the effect of blight and other forms of wheat damage upon cooking quality. The varieties were grown under comparable conditions at the two stations, while the damaged durum studies were made on carefully prepared blends of sound and damaged durum. This material has been described by Harris and Sibbitt (1942a,b) in some detail, with discussions of milling and macaroni-processing results and will, therefore, be but briefly touched upon in the present instance.

The cooking-quality study embraced 32 durum varieties grown at Fargo and Langdon. The Fargo wheats included 10 samples each of the 1939 and 1940 crops and Langdon supplied 12 varieties in 1940. The 1940 samples were practically all affected with blight and associated damage, especially the wheats grown at Langdon. No samples were available from Langdon for 1939, and accordingly, the results from this station would be somewhat vitiated by the effects of the fungus infections. The study connected with the injurious effects of fungus damage was carried out on

[1] Published with the approval of the director.
[2] Names of authors arranged alphabetically, no seniority is implied.

various experimental blends of damaged durum with sound uninjured wheat grown at Langdon in 1939. The damaged kernels were carefully separated from a representative sample of infected durum into two classifications according to the degree of apparent injury. "Light damage" included those kernels which showed injury only at the tip, while "heavy damage" comprised those evidencing damage in the crease and other portions of the kernel besides the tip. The different blends were varied from five to 75 per cent by weight of injured wheat.

DISCUSSION

The variety, grade, semolina protein, and cooking data for the 32 wheats grown at Fargo and Langdon are shown (Table 1). The durum varieties included in the table cover a wide range of quality, with Mindum and Kubanka representing wheats which have long been accepted as satisfactory for macaroni production; while Pentad, Monad, and Golden Ball are rejected because of unsatisfactory characteristics mainly related to color. Several new hybrids have been developed by the U. S. Bureau of Plant Industry [3] and have shown promise from the standpoint of color of the macaroni produced therefrom. These are indicated by a nursery number as they have not been named.

It will be noted that the amber durum wheats grown at Fargo all graded No. 1 hard amber durum, while those from Langdon were reduced in grade by fungus injury in 1940. The percentages of light, heavy, and total kernel damage are shown, reaching 19 per cent for one Mindum sample. The Kubanka grown under comparable conditions had only five per cent of damage and this was entirely light in degree. There is strong evidence of varietal resistance to these blights on durum wheat.[4] The semolina protein varied from 11.6 to 14.3 per cent, the Langdon semolinas being higher than the Fargo samples. The cooked-weight results ranged from 312.6 to 374.4 grams. These values are lower than the data reported by Binnington, Johannson, and Geddes (1939) and Harris and Knowles (1940).

The tenderness scores ranged from 90.1 to 111.1 with a general average of 96.3. These results are definitely lower than the values reported by Harris and Knowles (1940) on North Dakota durums from the 1938 crop, but the varieties tested in the different years varied greatly and it is difficult to make definite statements regarding annual changes in tenderness score. The presence of fungus damage tended, no doubt, to lower the scores in 1940, especially in the Langdon tests, as it will be shown later that damage of this nature decreases the tenderness score. The Langdon samples tend to give higher results than the Fargo durums, however, despite the damage. The same trend was noted by the authors in the 1938 crop wheats.

The yearly variations in cooked weight and tenderness score for four of the varieties which were grown consecutively during 1938, 1939, and

[3] Glenn Smith, Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, has been in direct charge of a breeding program instrumental in developing these hybrids.

[4] Information supplied by Glenn Smith.

TABLE 1

Comparative Cooking-Quality Data[1] With Associated Grades, Kernel Damage, and Semolina Protein From Various Durum Wheat Varieties

(Results arranged in order of increasing weight after cooking)

Sample No.	Variety	Unofficial grade[2]	Kernel damage: Light	Kernel damage: Heavy	Kernel damage: Total	Semolina protein (N×5.7)	Cooked weight	Residue	Tenderness score[3]
			pct.	pct.	pct.	pct.	gm.	pct.	
Fargo, 1939									
39-641	Kubanka	1 HAD	....	....	....	12.4	331.0	3.6	94.9
646	Kubanka 314	1 HAD	....	....	....	13.7	332.6	3.4	98.5
645	Kubanka 49	1 HAD	....	....	....	12.0	338.1	3.6	90.1
648	Ld 34	1 HAD	....	....	....	12.8	341.6	3.8	95.7
647	Kubanka 75	1 HAD	....	....	....	12.6	344.0	3.9	87.8
640	Mindum	1 HAD	....	....	....	12.5	345.8	4.1	95.1
644	Pentad	RD	....	....	....	12.1	346.0	4.2	92.0
643	Golden Ball	1 HAD	....	....	....	13.3	347.7	4.1	98.3
642	Monad	1 HAD	....	....	....	11.6	351.7	4.2	96.5
649	Ld 134	1 HAD	....	....	....	13.0	374.4	4.1	94.9
Average............	....	....	....	....	....	12.6	345.3	3.9	94.4
Fargo, 1940									
40-902	Kubanka 314	1 HAD	....	....	....	12.1	319.6	3.0	93.0
894	Kubanka	1 HAD	....	....	....	13.1	322.6	3.7	96.9
897	Ld 102	1 HAD	Some black point			12.7	322.8	3.6	94.1
896	Ld 34	1 HAD	Some black point			12.3	325.8	3.9	92.8
895	Pentad	RD	Some black point			12.5	327.2	3.4	96.4
899	Ld 111	1 HAD	Trace black point			12.2	327.4	3.4	101.2
900	Ld 134	1 HAD	Some broken kernels			12.9	329.0	3.1	95.9
898	Ld 104	1 HAD	Some black point			12.6	341.0	3.7	93.4
901	Kubanka 49	1 HAD	....	....	....	12.0	346.2	3.4	93.2
893	Mindum	1 HAD	Some black point			12.3	352.4	3.9	98.0
Average............	....	....	....	....	....	12.5	331.4	3.5	95.5
Langdon, 1940									
40-876	Ld 101	4 HAD	3	10	13	14.3	312.6	2.9	94.4
878	Ld 104	3 HAD	6	7	13	12.8	324.0	3.6	98.6
883	RL 1317	2 HAD	5	3	8	12.8	324.0	3.3	97.8
877	Ld 102	4 HAD	2	10	12	12.9	326.1	3.7	99.8
882	Ld 134	2 HAD	3	3	6	13.5	326.4	3.4	97.3
873	Kubanka	1 HAD	5	....	5	14.1	327.6	3.2	95.4
875	Ld 34	3 HAD	3	5	8	12.9	329.2	3.5	102.0
872	Mindum	3 HAD	12	7	19	12.6	330.6	3.6	97.7
880	Ld 111	5 HAD	5	12	17	12.4	331.0	4.0	111.1
874	Monad	1 HAD	6	2	8	12.6	336.6	3.3	95.1
881	Ld 133	3 HAD	5	5	10	13.1	336.8	3.2	96.6
879	Ld 105	3 HAD	4	6	10	12.8	337.8	3.8	96.2
Average............	....	....	....	....	....	13.1	328.6	3.5	98.5

[1] All results calculated on 100 grams of material containing 13.5 per cent moisture. [2] Key to grades: HAD = hard amber durum; RD = red durum.

[3] $\frac{\text{Time (in seconds) to break}}{\text{Time (in seconds) to .150 line}} \times 10 = \text{ratio}$; ratio + seconds to break + angle = tenderness score.

1940 at Fargo are listed (Table 2). The Langdon station is omitted since the 1939 samples were missing and the 1940 wheats from that location were more or less damaged by fungus injury.

It is apparent that yearly variations in these values exist, but that the varieties are affected in different ways in respect to these changes. The 1938 results were highest with one exception. The most satisfactory varieties for cooking quality were Ld 134, Mindum, and Kubanka 49 for Fargo, and Ld 105, Ld 133, and Monad for Langdon, in 1940.

TABLE 2

Annual Differences in Cooked Weight and Tenderness Score of Wheats Grown at Fargo

Year	Kubanka 314		Kubanka 49		Ld 34		Mindum	
	Cooked weight	Tenderness score	Cooked weight	Tenderness score	Cooked weight	Tenderness score	Cooked weight	Tenderness score
	gm.		*gm.*		*gm.*		*gm.*	
1938	382.0	98.0	381.4	98.0	354.1	119.1	357.7	96.7
1939	332.6	98.5	338.1	90.1	341.6	95.7	345.8	95.1
1940	319.6	93.0	346.2	93.2	325.8	92.8	352.4	98.0

In view of the results obtained by Harris and Knowles (1940) the correlation coefficients were calculated between cooked weight and semolina protein, cooked weight and tenderness score, and semolina protein and tenderness score (Table 3). It will be noted that the three coefficients are below the five-per cent level of significance. In working with the 1938 durum crop the authors found correlation coefficients of —.6227 between cooked weight and semolina protein, of —.4174 between cooked

TABLE 3

Correlation Coefficients Computed From the Data (N = 32)

Variables correlated		r_{xy}[1]
X	Y	
Cooked weight (gm.)	Semolina protein (pct.)	.3114
Cooked weight (gm.)	Tenderness score	.1803
Semolina protein (pct.)	Tenderness score	.1142

[1] Value of r_{xy} at five-per cent point = .349.

weight and tenderness score, and .7306 between semolina protein and tenderness score. The discrepancy in these findings probably lies in yearly variations in the properties of the durums examined. A further disturbing factor lies in the occurrence of fungus infections upon the durum crop in 1940, which no doubt altered the relationships among the variables under discussion.

The data obtained from the blends made with the light and heavy damaged durum were rather interesting (Table 4). The percentage by weight of the injured wheat is indicated with grade, semolina protein, cooked weight, residue, and tenderness score. The light damage was increased to a maximum of 75 per cent and the heavy to 50 per cent. The effect of heavy damage upon grade was very marked, the 50-per cent blend

reducing the rating to sample-grade durum. The apparent increase of semolina protein with damage is due to the higher protein content of the damaged-wheat sample. It is evident that damaged wheat consistently reduced the cooked weight and tenderness score, this effect being most marked when the wheat injury was severe. The amount of residue was increased by the infection with the heavily damaged kernels having the greatest effect. The cooking data in relation to amount of damage is shown graphically (Fig. 1). The changes, though small, are consistent and are no doubt significant. The effect of heavy damage in reducing cooked weight is the most important result revealed by this phase of the study.

TABLE 4

Comparative Cooking-Quality Data With Associated Grades and Semolina Protein From Blends Containing Graduated Proportions of Damaged Durum Wheat

(Arranged in order of increasing cooked weight within damage classification)

Sample No.	Blend description [1]	Unofficial grade [2]	Semolina protein (N×5.7)	Cooked weight [3]	Residue	Tenderness score [4]
			pct.	*gm.*	*pct.*	
40-906	Original sample (16% light, 12% heavy)	5 HAD	13.0	312.8	3.5	103.6
908	50% heavy	SGD	12.9	306.8	4.0	99.5
909	25% heavy	SGHAD	12.6	318.0	3.8	100.5
910	10% heavy	4 HAD	12.2	326.0	3.6	100.5
911	5% heavy	3 HAD	12.1	327.2	3.5	102.0
912	75% light	1 HAD	12.8	325.2	3.4	100.5
913	50% light	1 HAD	12.6	326.0	3.3	100.9
914	25% light	1 HAD	12.4	327.2	3.1	101.6
915	10% light	1 HAD	12.1	328.4	2.5	102.4
916	5% light	1 HAD	12.0	328.8	2.5	102.2

[1] Blends made with a sample of sound hard amber durum plus indicated percentage of damaged wheat. [2] Key to grades: HAD = hard amber durum; SGD = sample-grade durum; SGHAD = sample-grade hard amber durum. [3] All results calculated on 100 grams of material containing 13.5 per cent moisture. [4] See Footnote 3, Table 1.

SUMMARY AND CONCLUSIONS

Thirty-two samples of macaroni from various varieties of durum wheat produced at Fargo and Langdon in 1939 and 1940 were cooked by standardized methods. Cooked weight, amount of disintegration upon cooking, and tenderness of the cooked product were determined.

Varietal differences in cooked weight and tenderness score were demonstrated by the results obtained. Cooked-weight values were lower than those reported by other workers as well as by the authors in previous work. Yearly variations were also found for four varieties grown consecutively at Fargo for three years. Cooking quality apparently bears no relationship to color, the accepted criterion of macaroni value.

Correlation coefficients were computed between cooked weight and semolina protein, cooked weight and tenderness score, and semolina protein and tenderness score. These constants were below the level of significance and did not agree with the previous findings by the authors in which significant negative relationships were found between cooked weight and the other two variables, and a positive relationship between semolina protein and tenderness score. This may be explained by yearly differences

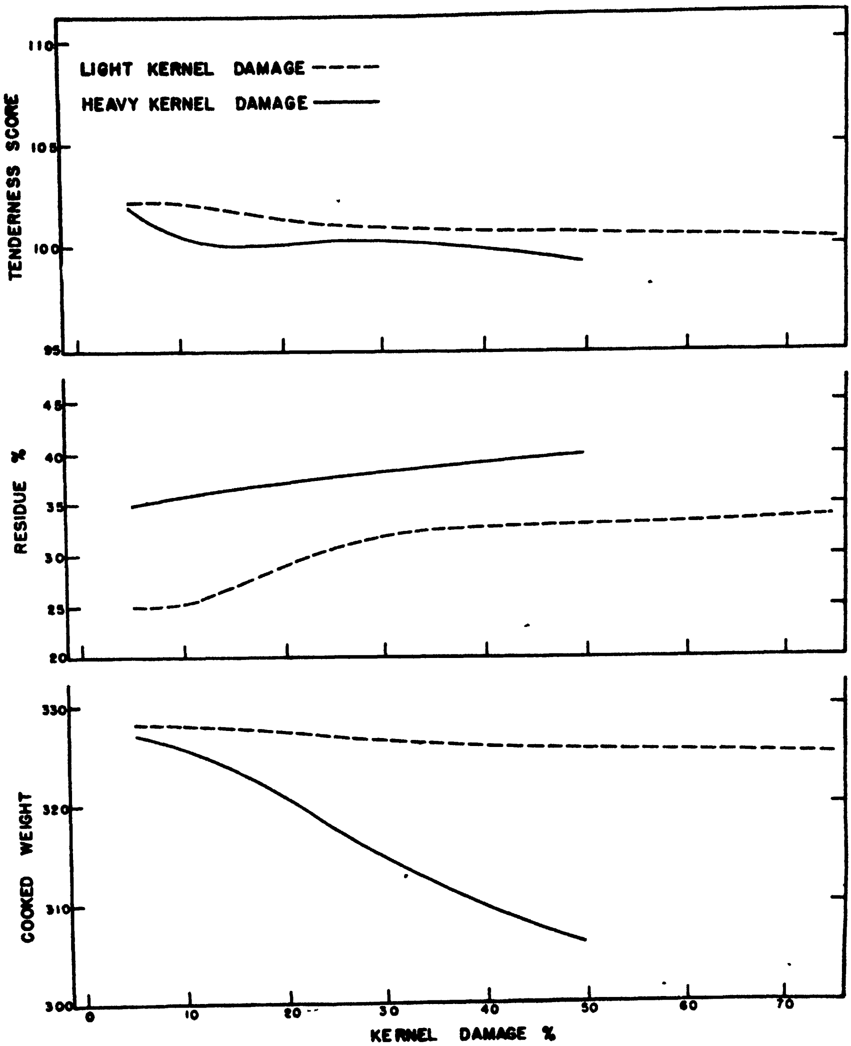

FIG. 1. Effect of light and heavy kernel damage upon cooked weight, residue, and tenderness score of cooked macaroni.

in properties among the durum varieties as well as by the effects of damage by wheat fungus.

Wheat blights, including the damage commonly known as "black point," were found to reduce significantly the cooked weight and tenderness score, particularly if the damage was severe. The degree of disintegration upon cooking was increased by the presence of infected wheat in the blend, heavily damaged kernels having the most marked effect.

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CAROTENE CONTENT OF FRESH AND FROZEN GREEN VEGETABLES[1]

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It is well known that certain fresh green vegetables constitute a significant source of vitamin A in human nutrition by virtue of their carotene content. It has also been well established that the carotene content varies widely among various plants and that carotene is frequently lost under some conditions of storage, DeFelice and Fellers (1938), Fitzgerald and Fellers (1938), Stimson, Tressler, and Maynard (1939), and Zimmerman, Tressler, and Maynard (1940, 1941). It is therefore of practical and of scientific interest to make a systematic comparison of the carotene contents of various vegetables as well as of different varieties of the same vegetable. In some cases it is also important to obtain information on the relative carotene contents of the various edible parts of the plant.

The changes in carotene content with maturity and during low-temperature storage are of practical importance owing to the increasing use of low-temperature storage of fresh-frozen foods. In 1941 the frozen-food industry did well over ten million dollars' worth of business, according to the H. J. Heinz Company (1941), and the volume is growing rapidly, as recently discussed by Waters (1942). Knowledge of nutritive value retained in stored foods is probably more important now than ever before. It is the purpose of this paper to compare the carotene contents of various vegetables and to show the changes in carotene content during low-temperature storage, following different methods of preparation for storage.

EXPERIMENTAL PROCEDURE

Method of Analysis: Beadle and Zscheile (1942) have described an accurate spectrophotometric method for the determination of the actual beta-carotene content of vegetables in which the principal carotene pigments are beta-carotene and its isomer neo-beta-carotene. They showed that the presence of neo-beta-carotene may explain the anomalous spectroscopic behavior of "carotene" observed by various workers. The influence of neo-beta-carotene on carotene analysis was discussed and it was noted that considerable error may be incurred if the presence of neo-beta-carotene is ignored.

All analyses reported in this paper were made in accordance with the method described by Beadle and Zscheile (1942) which may be summarized briefly as follows:

Five to 10 grams of spinach or broccoli sample were extracted for five minutes with 125 ml. of diacetone alcohol at room temperature in a War-

[1] Journal Paper No. 63 of the Purdue University Agricultural Experiment Station.

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ing blendor. For other vegetables, 20 grams were used with 150 ml. of diacetone alcohol. The diacetone alcohol was filtered off and the residue washed with diacetone alcohol, followed by approximately 15 ml. of hexane. The water content was adjusted, if necessary, so that there were 100 parts of diacetone alcohol to six parts of water. The carotene pigments were transferred to hexane by four successive extractions (25 ml. each) in a separatory funnel. (The first quantity of hexane used must be sufficient to cause the formation of two phases.) These extractions left the chlorophylls and carotenols in the aqueous diacetone alcohol except for traces which accompany the carotenes into the hexane. The hexane was then freed from the small amounts of chlorophylls and carotenols by washing with three portions (25 ml. each) of aqueous diacetone alcohol (100:6), followed by one washing with 25 ml. of 20 per cent potassium hydroxide in methanol. After washing the hexane solution of carotene thoroughly with water, it was dried over sodium sulfate and analyzed spectrophotometrically, using wave lengths 4360 A and 4780 A. A photoelectric spectrophotometer employing a double monochromator with crystal quartz optics was used. Application of a visual spectrophotometer to the analysis of such extracts has been discussed by Zscheile and Beadle (1942).

By this method it is possible to analyze the carotene fraction as a binary mixture, so that the beta-carotene and neo-beta-carotene values are obtained. The characteristic curve of the carotene fraction from spinach in hexane after saponification was the same whether the original extraction from the plant tissue was made with diacetone alcohol, acetone, or hexane (after drying the tissue overnight with solid sodium sulfate).

Samples and Treatment: Several varieties each of spinach, peas, green beans, and Lima beans were investigated. In addition to these, Martha Washington asparagus and market broccoli were studied. All the vegetables except the asparagus and broccoli were grown on a plot of land adjacent to the laboratory so that the fresh material could be analyzed as soon as removed from the plant. All fresh analyses (except market grade) were made in this way, so there was a minimum amount of time between the sampling and the actual analysis. The asparagus, being a perennial, was most conveniently obtained from an established commercial field and brought to the laboratory immediately by automobile. Broccoli was obtained from the local market.

Only the edible portions of the plants were analyzed. Spinach samples were classified according to leaf size. The leaves were considered small if they were one and one-half to two inches long, medium-sized if about three inches long, and large if four or more inches in length. Analyses were also made on leaves from plants which were chlorotic. In these samples, only the leaves which were visibly yellow or pale green were selected.

The peas were sampled according to the size of the pea after shelling. Immature peas were compared with fully formed peas. Samples were also obtained after the peas had become hard and starchy. Measurement of pea diameter did not have much meaning in comparisons of different varieties, for both the Alderman Tall Telephone and the Thomas Laxton varieties yield peas so much larger than the Alaska variety that the

diameter of an immature Laxton pea might well be the same as that of an Alaska pea too old to be edible.

Green beans were studied with relation to pod length. The entire pod is a common article of food and its length was thus a convenient measure of maturity. An exception is the Kentucky Wonder variety, in which case the pods were still tender and edible even when eight to 10 inches in length, while pods of the other varieties were tough and difficult to "snap" when six inches in length.

The Lima beans were graded according to shelled-bean size and color, and they were considered immature, medium, and past prime as the beans varied from the small, very green state to the larger, almost colorless condition. The Fordhook Bush variety produced very large beans which were graded without regard to the bean size of the other two varieties.

Asparagus presented a slightly different problem from the above vegetables. Analyses were made on samples composed of the entire edible portion of the stalk as well as of various parts of the stalk separately. The color of an asparagus cutting varies considerably from tip to base, the tip being fairly dark green and the base almost colorless. Commercially prepared asparagus is commonly sold either as "tips" or as complete cuttings. The tips were therefore analyzed in some instances as separate samples and compared with successive sections of the stalks immediately below on the same cuttings.

Broccoli was separated into leaves, flower tips, and stems.

The samples were put into low-temperature cold storage at the same time that the analyses of the fresh material were made. Certain samples were stored without blanching, in which case the material was put into glass jars and placed in a freezer at —20°C.(—4°F.). The samples which were blanched before placing in storage were immersed in boiling water for periods of time varying from 30 seconds to two minutes, depending on the type of material, then cooled in a cold-water bath and placed in glass jars at —20°C. All of the stored samples remained in the dark at —20°C. until analyzed at the intervals indicated.

The results of analyses give total carotene content as well as beta-carotene, on the fresh weight basis, with percentage losses of beta-carotene calculated separately (Table 1). Moisture contents are included to indicate the maturity of the sample and for calculation to the dry basis if desired.

An attempt was made to determine carotene losses during the processes of shelling, washing, blanching, and grading under commercial conditions. Analyses were subsequently carried out on the same samples after storage at —20°C. This was done for Henderson Lima beans, obtained from the Marion, Indiana, plant of the Snider Packing Corporation. Results are reported (Table 2) in which samples are listed in order as they were processed. All samples were placed in contact with dry ice immediately after removal from the processing line for transportation to the laboratory, where they were analyzed the next day. The all white Henderson Bush Lima beans represented all sizes, both green and white, whole, sound beans.

TABLE 1

Carotene Content of Vegetables[1]

Sample	Description	Storage time at —20°C.	Age from planting	Total Carotene	beta-Carotene		Loss of beta-Carotene	Moisture content
		mo.	*days*	*γ/gm.*	*pct.*	*γ/gm.*	*pct.*	*pct.*
	Spinach							
119	Giant Nobel, 1½", fr.	0	47	51.1	86.3	44.1	......	93
120	Giant Nobel, 3½", fr.	0	47	57.0	87.5	49.9	......	91
	3½", bl.	6	......	55.7	85.0	47.3	5.2	....
	3½", bl.	11	......	40.0	79.2	31.7	36.4	....
	3½", not bl.	6	......	34.1	76.0	25.9	48.1	....
	3½", not bl.	11	......	20.8	71.9	14.9	70.2	....
134	Giant Nobel, garden run, fr.	0	66	60.8	89.6	54.5	......	90
20	Giant Nobel, fr.	0	41	63.0	......	......	......	87
	bl.	12	......	39.9	80.0	32.0	36.7 t	92
	bl.	24	......	17.7	65.6	11.6	72.5 t	....
	not bl.	12	......	29.6	75.0	22.2	53.0 t	90
	not bl.	24	......	8.04	49.0	3.9	87.0 t	....
29	Giant Nobel, fr.	0	52	72.1	......	......	......	87
	bl.	12	......	55.0	83.3	45.8	23.7 t	94
	bl.	23	......	35.6	78.1	27.8	50.6 t	....
	not bl.	12	......	21.8	65.6	14.3	69.8 t	91
	not bl.	23	......	8.41	54.0	4.6	88.0 t	....
37	Giant Nobel, fr., 1½"	0	48	56.6	......	......	......	....
38	Giant Nobel, fr., 3½"	0	35	63.4	......	......	......	....
	bl.	12	......	56.6	87.5	49.5	10.7 t	93
	bl.	23	......	28.1	77.1	21.7	55.8 t	....
39	light colored	0	35	30.2	......	......	......	....
117	King of Denmark, 2", fr.	0	47	62.8	89.5	56.2	......	94
118	King of Denmark, 4", fr.	0	......	60.9	87.5	53.3	......	92
	4", bl.	6	......	58.2	85.2	49.6	6.9	....
	4", bl.	11	......	35.0	77.1	27.0	49.3	....
	4", not bl.	6	......	45.2	74.0	33.5	37.2	....
	4", not bl.	11	......	26.1	65.6	17.1	68.0	....
132	King of Denmark, garden run, fr.	0	66	60.2	89.6	54.0	......	91
22	King of Denmark, fr.	0	42	55.0	......	......	......	87
	bl.	12	......	39.6	84.5	33.5	28.0 t	91
	not bl.	12	......	26.6	70.4	18.7	51.6 t	90
46	King of Denmark, fr., 1½"	0	53	85.4	......	......	......	87
47	fr., 3½"	0	53	76.0	......	......	......	....
	bl.	12	......	59.2	81.3	48.2	22.1 t	90
	bl.	23	......	37.8	79.2	29.9	50.2 t	....
	King of Denmark, not bl.	12	......	34.1	66.7	22.7	55.1 t	87
	not bl.	23	......	22.4	77.1	17.3	70.5 t	....
48	King of Denmark, light colored, fr.	0	53	29.1	......	......	......	....

121	Va. Blight Res., 1½", fr.	0	47	87.0	86.0	74.8	......	94
122	Va. Blight Res., 3½", fr.	0	47	69.0	87.5	60.4	......	90
	3½", bl.	6	......	67.0	85.4	57.2	5.3	....
	3½", bl.	12	......	40.1	74.0	29.7	50.9	....
	3½", not bl.	6	......	38.0	74.0	28.1	53.5	....
	3½", not bl.	12	......	22.5	64.6	14.5	76.0	....
133	Va. Blight Res., garden run	0	66	80.7	91.7	73.9	......	92
23	Va. Blight Res., fr.	0	41	86.0	......	......	......	86
	bl.	12	......	62.7	83.4	52.3	27.1 t	91
	bl.	23	......	38.7	69.8	27.0	55.0 t	....
	not bl.	12	......	49.2	77.0	37.9	42.8 t	88
	not bl.	23	......	14.3	48.0	5.9	83.0	....
30	Va. Blight Res., fr.	0	51	62.5	......	......	......	86
	bl.	12	......	61.0	82.3	50.3	2.4 t	93
	bl.	23	......	40.0	76.0	30.4	36.0 t	....
	not bl.	12	......	31.2	75.0	23.4	50.1 t	93
	not bl.	23	......	11.5	54.0	6.2	81.0 t	....
44	Va. Blight Res., fr., 1½"	0	53	86.2	......	......	......	....
	bl.	12	......	49.0	80.2	39.3	43.2 t	91
	bl.	23	......	43.4	79.2	34.3	49.6 t	....
45	Va. Blight Res., fr., 3½"	0	53	73.1	......	......	......	87
49	light colored, fr.	0	......	30.9	......	......	......	....
	Asparagus							
113A	Asparagus tips, fr.	Market	3 27-41	7.70	79.2	6.10	......	....
B	Asparagus stalk, 2"	Market	3 27-41	5.40	75.0	4.05	......	....
114A	Martha Washington top, 4", young, fr.	0	5-3-41	9.51	82.3	7.83	......	89
B	Martha Washington top, 4", medium, fr.	0	5-3-41	8.67	83.9	7.28	......	88
	top, 4", bl.	8	......	6.70	77.1	5.17	29.0	....
	top, 4", bl.	13	......	4.21	66.7	2.81	61.5	....
116A	Martha Washington, medium size	0	5-7-41	7.12	83.0	5.91	......	90
	medium size, bl.	8	......	5.57	75.0	4.18	29.3	....
	medium size, not bl.	8	......	2.28	65.6	1.50	74.7	....
	medium size, not bl.	13	......	1.65	59.0	0.97	83.0	....
114D	Martha Washington top, thick stalk	0	5-3-41	5.15	81.3	4.19	......	90
E	Martha Washington, light colored	0	5-3-41	4.11	80.2	3.29	......	90
F	Martha Washington, dark colored	0	5-3-41	9.35	79.7	7.47	......	90
31	Martha Washington top, 3", fr.	0	6-29-40	7.36	......	......	......	90
	not bl.	12	......	<0.25	......	......	100	93
35	Martha Washington tips, fr.	0	7-6-40	16.5	......	......	......	86
	tips, bl.	12	...	8.45	80.2	6.78	48.8 t	91
	tips, bl.	23	......	4.97	71.9	3.58	70.0 t	....
36	Martha Washington, stem below tip, fr.	0	......	7.09	......	......	......	90
52	Martha Washington tips	0	7-16-42	11.9	......	......	......	....
53	Martha Washington, upper stem, 2"	0	......	4.91	......	......	......	....
54	Martha Washington, lower stem, 2"	0	......	2.91	......	......	......	....

TABLE 1 (*Continued*)

Carotene Content of Vegetables[1]

Sample	Description	Storage time at —20°C.	Age from planting	Total carotene	beta-Carotene		Loss of beta-Carotene	Moisture content
		mo.	*days*	*γ/gm.*	*pct.*	*γ/gm.*	*pct.*	*pct.*
	Broccoli							
115A	Broccoli leaves, fr.	Market	5-10-41	97.5	89.6	87.4	......	89
B	Broccoli tips, fr.	Market	5-10-41	29.4	82.3	24.2	......	85
C	stems, 4", fr.	Market	5-10-41	6.11	84.0	5.13	......	93
D	Broccoli, whole sample, fr.	Market	5-10-41	25.0	85.4	21.3	......	90
	whole sample, bl.	7	......	16.8	75.0	12.6	40.8	....
	whole sample, bl.	12	......	19.4	77.1	15.0	29.6	....
	Beets							
131	Beet leaves, fr.	?	7-3-41	51.1	89.6	45.8	......	....
	Peas							
123	Alaska, medium, fr.	0	46	4.10	85.4	3.50	......	73
	medium, not bl.	6	......	1.69	65.0	1.10	68.7	....
	medium, not bl.	12	......	0.58	27.0	0.16	95.0	....
126	Alaska, small, fr.	0	57	4.05	86.5	3.50	......	73
	small, bl.	6	......	5.90	76.5	4.51	—28.8	....
	small, bl.	11	......	5.10	68.7	3.50	0	....
142	Alaska, past prime, fr.	0	48	5.13	83.3	4.28	......	67
	past prime, bl.	6	......	4.77	86.0	4.10	4.21	....
	past prime, bl.	13	......	5.19	75.0	3.89	9.34	....
	past prime, not bl.	13	......	3.79	74.0	2.80	34.6	....
127	Alaska, large, fr.	0	58	6.06	84.4	5.12	......	74
141	Alaska, medium, fr.	0	41	4.65	82.3	3.83	......	....
24	Alaska, fr.	0	43	4.23	......	......	......	....
27	Alaska, fr.	0	49	5.46	......	......	......	76
	bl.	12	......	4.16	81.1	3.38	23.8 t	74
	bl.	23	......	2.59	62.5	1.62	52.5 t	....
	not bl.	12	......	2.21	79.2	1.75	59.4 t	76
	not bl.	23	......	0.11	......	0	98.0 t	....
32	Alaska, fr.	0	43	4.83	......	......	......	....
	bl.	12	......	4.79	81.3	3.90	0.8 t	76
128	Thos. Laxton, small, fr.	0	59	4.80	81.3	3.91	......	74
	small, bl.	6	......	5.43	76.5	4.15	—6.14	....
144	Thos. Laxton, past prime, fr.	0	49	4.50	80.2	3.61	......	75
	past prime, bl.	6	......	5.72	85.4	4.88	—35.2	....
	past prime, bl.	13	......	4.22	72.9	3.08	14.7	....
145	Thos. Laxton, past prime, fr.	0	72	3.86	77.6	3.00	......	72
	past prime, bl.	6	......	3.89	80.2	3.12	—4.0	....
	past prime, bl.	13	......	3.11	72.9	2.27	24.3	....
143	Thos. Laxton, medium, fr.	0	49	4.64	81.3	3.77	......	....
129	Thos. Laxton, large, fr.	0	58	5.30	82.3	4.36	......	78

28	Thos. Laxton, fr.	0	49	5.27	......	......	......	75
	bl.	12	......	3.66	75.0	2.75	30.6 t	82
	bl.	23	......	2.05	56.0	1.1	61.0 t	....
	not bl.	12	......	0.87	70.2	0.61	83.5 t	81
	not bl.	23	......	0	......	0	100 t	....
139	Alderman T. T., medium, fr.	0	71	4.73	79.2	3.75	......	....
140	Alderman T. T., past prime, fr.	0	71	3.85	82.3	3.17	......	77
	past prime, bl.	6	......	4.72	85.4	4.03	—27.2	....
	past prime, bl.	13	......	4.31	75.0	3.23	—1.9	....
	past prime, not bl.	6	......	2.66	78.1	2.08	34.4	....
	past prime, not bl.	13	......	1.80	68.7	1.24	60.8	....
43	Alderman T. T., fr.	0	67	5.06	......	......	......	70
	bl.	12	......	4.20	78.1	3.28	17.0 t	71
	bl.	23	......	4.07	70.8	2.88	20.0 t	....
	not bl.	12	......	2.30	77.1	1.77	54.5 t	75
	not bl.	23	......	1.91	70.8	1.35	62.2 t	....
42	Alderman T. T., fr.	0	72	5.32	......	......	......	77
	Beans							
138	Bountiful, 5", fr.	0	67	3.00	79.2	2.38	......	91
	5", bl.	6	......	1.62	75.0	1.21	49.2	....
	5", bl.	13	......	1.88	69.8	1.31	45.0	....
	5", not bl.	6	......	0.60	60.8	0.36	84.8	....
	5", not bl.	13	......	0.36	37.0	0.13	95.0	....
147	Bountiful, 5", fr.	0	49	1.98	70.3	1.39	......	94
	5", bl.	6	......	1.58	70.8	1.12	19.4	....
	5", bl.	13	......	1.25	65.6	0.82	41.0	....
	5", not bl.	6	......	0.42	42.0	0.18	87.0	....
	5", not bl.	13	......	0.25	35.0	0.09	94.0	....
137	Bountiful, 2½", fr.	0	67	6.90	70.8	4.88	......	90
146	Bountiful, 3", fr.	0	49	5.38	59.0	3.20	......	....
33	Bountiful, 3", fr.	0	52	5.98	......	......	......	89
34	Bountiful, 6", fr.	0	......	3.49	......	......	......	91
	6", bl.	12	......	2.31	71.9	1.66	33.9 t	92
	6", bl.	23	......	0.88	71.9	0.63	74.8 t	....
	6", not bl.	12	......	<0.25	......	0	100 t	....
50	Bountiful, past snap size, fr.	0	62	1.58	......	......	......	86
59	Bountiful, 2½", fr.	0	48	12.0	......	......	......	88
60	Bountiful, 5", fr.	0	......	5.03	......	......	......	88
136	Asgrow, 5", fr.	0	67	3.40	79.2	2.69	......	90
	5", bl.	6	......	2.82	76.5	2.16	19.7	....
	5", bl.	13	......	2.63	70.8	1.86	30.9	....
135	Asgrow, 2½", fr.	0	67	6.40	74.0	4.73	......	89
40	Asgrow, 2½", fr.	0	55	7.30	......	......	......	90
41	Asgrow, 5", fr.	0	......	3.54	......	......	......	91
	5", bl.	12	......	2.28	67.7	1.54	35.5 t	91

TABLE 1 (*Concluded*)

Carotene Content of Vegetables[1]

Sample	Description	Storage time at —20°C.	Age from planting	Total carotene	beta-Carotene		Loss of beta-Carotene	Moisture content
		mo.	*days*	*γ/gm.*	*pct.*	*γ/gm.*	*pct.*	*pct.*
	5", bl.	23	......	1.86	70.8	1.32	47.5 t	....
	5", not bl.	12	......	0.86	63.5	0.55	75.8 t	91
	5", not bl.	23	......	0	......	0	100 t	....
51	Asgrow, past snap size, fr.	0	61	1.86	......	......	......	85
61	Asgrow, 2", fr.	0	48	14.4	......	......	......	88
62	Asgrow, 5", fr.	0	......	3.96	......	......	......	89
149	Kentucky Wonder, 5", fr.	0	75	5.30	76.0	4.03	......	92
	5", bl.	6	......	4.85	77.1	3.74	7.2	....
	5", bl.	12	......	3.04	72.9	2.22	45.0	....
150	Kentucky Wonder, 9", fr.	0	75	3.90	75.0	2.92	......	93
	9", bl.	6	......	4.00	77.1	3.08	—5.5	....
	9", bl.	13	......	2.58	67.7	1.75	40.0	....
	9", not bl.	6	......	0.54	42.0	0.22	92.0	....
	9", not bl.	13	......	0.37	42.0	0.16	95.0	....
157	Kentucky Wonder, 9", wilted, fr.	0	71	2.97	76.0	2.26	......	91
	9", wilted, bl.	5	......	2.67	75.0	2.00	11.5	....
	9", wilted, bl.	12	......	1.97	64.6	1.27	43.8	....
	9", wilted, not bl.	5	......	0.98	53.0	0.52	77.0	....
	9", wilted, not bl.	12	......	0.42	44.0	0.18	92.0	....
148	Kentucky Wonder, 3", fr.	0	75	6.24	71.9	4.48	......	91
153	Kentucky Wonder, 6", fr.	0	66	4.21	66.7	2.81	......	....
155	Kentucky Wonder, 3", wilted, fr.	0	71	10.0	73.4	7.34	......	93
156	Kentucky Wonder, 6", wilted, fr.	0	71	4.75	75.0	3.56	......	88
56	Kentucky Wonder, 2½", fr.	0	66	15.0	......	......	......	88
57	Kentucky Wonder, 4", fr.	0	68	8.17	......	......	......	88
58	Kentucky Wonder, 6", fr.	0	68	4.48	......	......	......	89
64	Kentucky Wonder, 8", fr.	0	72	4.71	......	......	......	86
	Kentucky Wonder, 8", bl.	12	......	1.86	62.5	1.16	60.4 t	91
	Kentucky Wonder, 8", bl.	23	......	1.30	65.6	0.85	72.5 t	....
	Kentucky Wonder, 8", not bl.	12	......	<0.25	......	0	...... t	89
	Kentucky Wonder, 8", not bl.	23	......	0	......	0	100 t	....
	Lima beans							
162	Baby Potato, medium, fr.	0	103	4.04	70.8	2.86	......	67
	medium, bl.	5	......	1.52	63.5	0.96	66.5	....
	medium, bl.	12	......	1.03	50.0	0.51	82.0	....
	Baby Potato, medium, not bl.	5	......	1.34	65.6	0.87	69.8	....
	medium, not bl.	12	......	0.627	53.0	0.33	88.0	....
189	Baby Potato, medium, fr.	0	90 (late)	0.88	69.8	0.61	......	63
	medium, not bl.	5	......	0.83	64.6	0.53	13.1	....

	medium, not bl.	11	......	0.63	53.0	0.33	46.0	....
190	Baby Potato, large, fr.	0	90(late)	0.32	62.0	0.19	......	60
89	Baby Potato, small, fr.	0	113	1.77	......	......	......	73
	small, bl.	12	......	1.54	48.0	0.74	13.0 t	72
90	Baby Potato, past prime, fr.	0	113	0.54	......	......	......	58
161	Baby Potato, small, fr.	0	103	4.75	62.5	2.97	......	75
163	Baby Potato, large, fr.	0	103	1.05	53.0	0.56	......	62
188	Baby Potato, small	0	90(late)	2.22	69.1	1.54	......	72
67	Fordhook Bush, small, fr.	0	59	5.70	......	......	......	....
91	Fordhook Bush, medium, fr.	0	127	1.87	......	......	......	74
92	Fordhook Bush, large, fr.	0	128	0.56	......	......	......	66
169	Fordhook Bush, small, fr.	0	103	3.45	65.6	2.26	......	70
170	Fordhook Bush, medium, fr.	0	103	1.76	64.0	1.13	......	65
	Fordhook Bush, medium, bl.	5	......	0.87	59.0	0.51	54.0	....
	medium, bl.	12	......	0.397	48.0	0.19	83.0	....
	medium, not bl.	5	......	0.71	50.0	0.35	69.0	....
	medium, not bl.	12	......	0.49	49.0	0.24	79.0	....
171	Fordhook Bush, large, fr.	0	103	1.07	63.7	0.68	......	66
191	Fordhook Bush, small, fr.	0	90	2.72	68.8	1.87	......	73
192	Fordhook Bush, medium, fr.	0	90	0.85	67.7	0.57	......	62
	Fordhook Bush, medium, not bl.	11	......	0.52	48.0	0.25	56.0	....
193	Fordhook Bush, large, fr.	0	90	0.57	63.6	0.36	......	59
	Fordhook Bush, large, not bl.	11	......	0.21	32.0	0.07	81.0	....
158	Henderson Bush, small, fr.	0	94	4.78	67.7	3.24	......	75
159	Henderson Bush, medium, fr.	0	94	4.16	72.9	3.03	......	68
	medium, bl.	5	......	1.79	62.5	1.12	63.0	....
	medium, bl.	12	......	1.70	54.0	0.92	70.0	....
	medium, not bl.	5	......	1.41	61.0	0.86	71.7	....
	medium, not bl.	12	......	1.00	51.0	0.51	83.0	....
160	Henderson Bush, large, fr.	0	94	1.24	62.5	0.77	......	62
166	Henderson Bush, small, fr.	0	81	2.94	65.6	1.93	......	77
167	Henderson Bush, medium, fr.	0	81	2.58	70.8	1.82	......	67
	medium, bl.	12	......	0.90	58.0	0.52	71.0	....
	medium, not bl.	5	......	1.17	65.6	0.77	57.7	....
	medium, not bl.	12	......	0.58	54.0	0.31	83.0	....
168	Henderson Bush, large, fr.	0	81	1.14	60.4	0.69	......	59

[1] In Tables 1 to 4, fr. signifies "fresh," bl. signifies "blanched," and not bl. signifies "not blanched." Dimensions of vegetable parts are given in inches. Under "age from planting," a date signifies date of picking or purchase. Moisture contents were determined by drying 10 gram samples overnight in an air oven at 105°C.(221°F.).

In most cases total carotene pigments were calculated from observations at 4360A, and the percentage beta-carotene from those at 4780A. In Tables 1 and 2 some samples were analyzed in 1940 for total carotene at wave lengths other than 4360A before the analytical scheme for the beta-carotene-neo-beta-carotene system had been developed. No percentage beta-carotene is reported for these cases. To obtain total carotene values for comparison with later, more accurate analyses, the assumption was made that the percentage beta-carotene was the same as the average values found in 1941 for the corresponding vegetable. The values obtained are listed as of 4360A for comparative purposes. Thus under "loss of beta-carotene," when the number is followed by t, loss of total carotene is given with no calculation of beta-carotene separately because of insufficient spectroscopic information when the sample was fresh.

TABLE 2

Carotene Content of Henderson Bush Lima Beans During and After Processing for Quick-Freezing[1]

Sample	Description	Storage time at —20°C.	Age from planting	Total carotene	beta-Carotene		Loss of beta-Carotene	Moisture content
		mo.	*days*	*γ/gm.*	*pct.*	*γ/gm.*	*pct.*	*pct.*
	Not graded							
83	Henderson Bush, from truck, fr.	0	9-16-40	1.22	......	....	......	55
84	Henderson Bush, shelled, fr.	0	9-16-40	1.20	......	....	......	53
	shelled, not bl.	12	9-16-40	1.08	62.0	.67	10.0 t	53
84a	Henderson Bush, washed, fr.	0	9-16-40	0.93	......	....	......	57
	washed, not bl.	12	9-16-40	0.80	61.5	.49	14.0 t	56
	washed, not bl.	22	9-16-40	0.52	52.0	.27	44.0 t	....
85	Henderson Bush, bl., fr.	0	9-16-40	0.42	......	....	......	61
	bl.	12	9-16-40	0.32	56.0	.18	24.0 t	61
	bl.	22	9-16-40	0.42	44.0	.18	0.0 t	....
	Graded							
86	All white Henderson Bush Lima beans	0	9-16-40	0.29	......	....	......	56
	All white Henderson Bush Lima beans	12	9-16-40	0.28	31.0	.08	3.5 t	55
87	Green and white Henderson Bush Lima beans	0	9-16-40	0.68	......	....	......	63
	Green and white Henderson Bush Lima beans	12	9-16-40	0.76	50.0	.38	—12.0 t	62
88	All green Henderson Bush Lima beans	0	9-16-40	1.32	......	....	......	70
	All green Henderson Bush Lima beans	12	9-16-40	1.51	53.0	.80	—14.0 t	69

[1] See footnote, Table 1.

The green and white consisted of small sizes of both green and white beans. The all green included only the smaller green and most tender beans.

It was considered advisable to study a single sample of spinach with extreme care, making analyses at frequent intervals during the early portion of the storage period. Two pounds of good, fresh, market spinach were purchased locally. The midribs were cut from the leaves, which were then finely shredded with shears. After thorough mixing of the shredded material, 10-gram samples were weighed out, the leaves being stirred between each sampling. Seventeen samples were used raw, and the

TABLE 3

Carotene Content of Raw and Cooked Spinach During Storage Period at —20° C.(—4° F.)[1]

Sample	Description	Storage time	Total carotene	Beta-carotene		Loss of beta-carotene
		days	*γ/gm.*	*pct.*	*γ/gm.*	*pct.*
206A	Fresh	0	55.6	88.5	49.2	......
B	Fresh (duplicate)	0	54.4	89.5	48.8	......
D	Not blanched	3	56.6	85.5	48.4	0
E	Not blanched (duplicate)	3	56.7	86.5	49.1	0
F	Not blanched	6	55.8	83.3	46.5	5.1
G	Not blanched (duplicate)	6	55.1	82.3	45.4	7.4
H	Not blanched	20	51.0	84.0	42.8	12.7
I	Not blanched (duplicate)	20	50.6	84.0	42.6	13.0
K	Not blanched	27	49.5	81.2	40.2	18.0
L	Not blanched (duplicate)	27	50.6	80.2	40.6	17.2
M	Not blanched	34	50.6	83.3	42.2	13.9
N	Not blanched (duplicate)	34	50.6	83.3	42.2	13.9
O	Not blanched	56	46.1	77.1	35.5	27.5
P	Not blanched	85	44.8	79.2	35.5	27.5
Q	Not blanched	210	18.4	70.8	13.0	73.4
R	Not blanched	330	9.5	72.4	6.9	86.0
T	Not blanched	354	8.8	67.2	5.9	88.0
C	Cooked	0	60.6	86.5	52.4	......
J	Cooked	20	59.4	83.0	49.4	5.7
S	Cooked	330	54.4	83.3	45.3	13.6
U	Cooked	354	51.2	80.2	41.0	21.8

[1] See footnote, Table 1.

last four were cooked for 30 minutes in a steam bath. The first two samples taken were analyzed immediately as raw controls, and the last sample taken was analyzed as a cooked control; results are reported (Table 3).

A few vegetable samples were analyzed for chlorophyll by the method of Comar and Zscheile (1942) in addition to carotene to determine the chlorophyll-carotene ratios; results are shown (Table 4).

DISCUSSION

From the results (Table 1) it may be concluded in general that blanched samples retain carotene much better during low-temperature cold storage than do those which are not blanched before freezing. This is an excellent reason for continuation of the commercial practice of blanching. No generalization is possible regarding the carotene contents

of younger vegetable tissue compared with those of corresponding larger and more mature vegetables, although in both snap beans and Lima beans, the carotene content decreases rapidly as maturity is approached. Intervarietal differences were quite small in all cases. The percentage of beta-carotene was fairly constant in all the fresh vegetables excepting beans and Lima beans in which this percentage was uniformly low. The percentage of beta-carotene decreased during low-temperature cold storage and often decreased more rapidly in samples that were not blanched than in blanched ones. Correspondingly, the percentage loss of beta-carotene was usually less for the blanched samples. These vegetables may be classified according to carotene content as high (spinach and broccoli), medium (asparagus, peas, and beans) and low (Lima beans). In the case of

TABLE 4

Chlorophyll-Carotene Ratios in Fresh Green Vegetables[1]

Vegetable	Total carotene	Beta-carotene	Total chlorophyll	Chlorophyll *a*	Chlorophyll-carotene ratio
	γ/gm.	pct.	γ/gm.	pct.	
Asparagus, light green	4.11	80.2	46.7	71.6	11.4
Asparagus, dark green	9.35	79.7	69.0	72.6	7.4
Peas, Alaska	4.05	86.5	51.9	69.4	12.8
Spinach, King of Denmark	60.20	89.6	854.0	72.0	14.2
Green beans, Kentucky Wonder	4.21	66.7	107.0	66.5	25.4
Lima beans, Baby Potato	4.04	70.8	51.8	69.4	12.8

[1] See footnote, Table 1.

blanched spinach, losses are small during the first six months of low-temperature cold storage but appreciable after 12 months and much greater after two years. When not blanched, the loss after six months is about equal to that of blanched spinach after one year; the loss after 12 months is considerable and after two years it becomes very great. Blanching is therefore most effective during the first portion of the storage period. Similar considerations probably apply to the other vegetables but they cannot be demonstrated so well, either because of the much smaller initial carotene content or because of sampling difficulties.

The following remarks apply to individual vegetables and are occasionally exceptions to the above general statements.

Spinach: This vegetable has the highest carotene content (total and beta-carotene) of any vegetable yet studied in this laboratory, though it is equaled or exceeded by the leaves of certain forage crops. Even light-colored, chlorotic samples, though lower than normal green samples, are relatively high. The carotene content of fresh spinach was much lower than reported by DeFelice and Fellers (1938), who used a very different method for determination of carotene. Losses on freezing are also much smaller than they reported.

Asparagus: The sampling error is large owing to the mixture of stem and leaf bracts in the edible portion. The tips are uniformly higher in carotene than the stalks, and light-colored sprouts are lower than darker green ones. Sample 114D was an abnormal type with a very flat but

thick, vigorous sprout. Losses of carotene were higher after eight months than was the case for spinach.

Broccoli: Sampling errors are very large in this case owing to the heterogeneous structure of the edible portion (flower, leaf, and stem). Leaves are very high, tips lower, and stems are low, giving high average values. Losses are appreciable after seven months of low-temperature cold storage.

Peas: Blanched samples often showed an increase in total carotene after storage, causing apparent gains or negative losses of beta-carotene. This may have resulted from better extraction after blanching and storage. It should be pointed out that the amounts of carotene involved are rather small for this vegetable and small amounts of colored decomposition products would seriously affect analytical results. The results of Stimson, Tressler, and Maynard (1939) on Thomas Laxton peas agree quite well with results reported here in so far as amounts found in fresh and frozen samples and loss during 11 months' storage at —20° C.(—4° F.) are concerned.

Beans: Younger, shorter beans are always higher in carotene content than older and longer ones. These findings are similar to those of Snyder and Moore (1940) with certain forage crops. The percentage of beta-carotene is definitely lower than in the case of the other vegetables except Lima beans. The carotene contents of snap beans reported by Zimmerman, Tressler, and Maynard (1940) are comparable to those reported here.

Lima beans: This vegetable has a very low total carotene content as well as a comparatively low percentage of beta-carotene. Smaller and younger beans are invariably higher than larger, older ones. The amounts of carotene involved are so small as to render such analyses of little practical significance.

Values reported here are considerably lower than the upper ranges found by Fitzgerald and Fellers (1938) for spinach, green peas, and Lima beans. Their preliminary work indicated no appreciable loss of pro-vitamin A during processing and quick-freezing. Values for spinach and beans are similar to those reported by Fraps, Meinke, and Kemmerer (1941). Values for fresh asparagus and Lima beans are in substantial agreement with results of Zimmerman, Tressler, and Maynard (1941).

It is apparent (Table 2) that losses occur during washing and blanching of Lima beans and that large differences exist between the three grades. However, the amounts of carotene present are insufficient to make the Lima bean of practical importance as a source of pro-vitamin A, and the causes of the apparent gains of beta-carotene are not worth further investigation for this vegetable.

Comparison of the raw and cooked fresh samples (Table 3) shows the difficulty of sampling, since it has been shown by Beadle and Zscheile (1942) that in other samples of spinach, extraction is equally good in the two cases. The duplicate successive samples were in excellent agreement throughout this study. Among the raw samples it is evident that losses did not become excessive until after the 34th day, after which deterioration was rapid. Losses were negligible for the first three days, which provides a convenient period during which samples may be stored fresh

before analysis. A progressive decrease in percentage beta-carotene is noted. Losses in the cooked samples were considerably less than in the raw ones. Although Sample 206S, stored 330 days after cooking, was brown in color and gave a brown extract, owing in part to chlorophyll decomposition, the characteristic curve of the carotene fraction analyzed well as a mixture of beta-carotene and neo-beta-carotene between 4360 and 4900A. The raw samples remained bright green to the end of the experiment.

Approximately the same range was found for total chlorophyll as for total carotene (Table 4). Small differences exist in the values for percentage chlorophyll *a*. The chlorophyll-carotene ratio was essentially constant for four samples, but obviously high chlorophyll does not necessarily indicate a correspondingly high carotene content in comparison of different vegetables, though this trend is indicated by results reported here when different samples of the same vegetable are compared.

During the progress of analysis it was noted that low percentages of beta-carotene were usually associated with low total-carotene contents. Spectroscopic observations were usually made at 4850A as well as at 4780A. When lower percentage beta-carotene values were obtained following long storage, agreement between these wave lengths usually became poor, indicating the decomposition of carotene to pigments of unknown identity. For this reason, analyses on very old or very deteriorated samples are often inexact, especially on unblanched samples.

It has been stated by Zimmerman, Tressler, and Maynard (1941) that the carotene fraction of green beans and Lima beans consists of about one-third alpha-carotene and two-thirds beta-carotene. This conclusion was based on chromatographic evidence for identification and upon spectroscopic data which were rather inconclusive because their observations were made before the possible presence of neo-beta-carotene was considered and before our most accurate spectroscopic standards had been determined, according to Zscheile, White, Beadle, and Roach (1942). A re-examination of these data in the light of later information indicates that the relative amounts of alpha-carotene present in these two vegetables are quite small.

SUMMARY

A study was made of the carotene contents of spinach, asparagus, broccoli, beet leaves, peas, green beans, and Lima beans. Analyses were made spectrophotometrically for total carotene as well as for beta-carotene and neo-beta-carotene. Vegetables were analyzed at different stages of development and after various periods (up to two years) of low-temperature cold storage after quick-freezing. In many cases, blanched samples were compared with unblanched ones.

The practice of blanching before quick-freezing is good because it retards the loss of carotene. It is most effective during the first year of storage.

Varietal differences were small.

During storage both the total carotene and the percentage of beta-carotene decreases.

During commercial processing of Lima beans, preparatory to quick-freezing, some losses occur. Various commercial grades differ widely in carotene content.

Fresh raw spinach may be kept frozen for 34 days without large losses of carotene.

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SYNTHESIS OF VITAMINS BY MICROORGANISMS IN RELATION TO VITAMIN CONTENT OF FANCY CHEESES

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Our knowledge concerning the occurrence and origin of vitamins in fancy cheese is relatively meager, although such information is highly desirable in view of the fact that cheese products are rapidly coming into greater prominence as substitutes for meat. Great differences in vitamin content of various kinds of cheese have been reported by the few investigators who have studied the problem, Booher, Hartzler, and Hewston (1942).

In general the vitamin content of cheese depends upon the amount present in the original milk, the methods employed in forming the fresh curd cakes, and the subsequent history of the cheese through the various processes of curing up to the time when the product is consumed. To what extent may the bacteria, yeasts, or molds, which are present in large numbers during the stages of ripening, influence the vitamins stored in fancy cheeses?

Day and Darby (1936) observed large differences in riboflavin content of several kinds of cheese. Randoin and Raffy (1941) reported very high values for vitamin B_2 in certain French cheeses. Sullivan, Bloom, and Jarmol (1943) assayed vitamin B_2, pantothenic acid, nicotinic acid, and biotin in 12 varieties of cheese by means of microbiological procedures. Appreciable increases were observed in the pantothenic acid, niacin, and biotin content of Limburger cheese studied through progressive stages of curing. Different kinds of cheese varied greatly in their content of B vitamins, depending upon the techniques of preparing the curd and the nature of the ripening processes employed.

EXPERIMENTAL PROCEDURE

The investigations reported in this paper were concerned with Camembert, Liederkranz, Brie, and Limburger cheeses which were sampled at several progressive stages of curing. The cheeses were generously supplied by G. C. Supplee, Wilhelmine van Wieren, and G. W. Wilson of the Borden Company. A number of dominant microorganisms were isolated from each of the ripened cheeses for the study of growth habits and determination of vitamins produced by the yeasts, molds, and bacteria cultured in chemically defined media.

In view of certain preliminary observations on the vitamin content of the superficial layers of fancy cheeses containing numerous microorganisms, it seemed desirable to inquire into the possibility that these microorganisms might produce and store vitamins in the medium supporting their growth. Yeasts, molds, and bacteria were isolated and carried as stock cultures on nutrient agar. Two additional organisms commonly found in dairy

products were supplied by the Department of Bacteriology, University of Wisconsin. The organisms were studied in chemically defined media and in media enriched with liver concentrate according to the methods described in an earlier paper by Burkholder (1943).

The observed growth responses and vitamin requirements of the organisms (Table 1) indicate that many of them are capable of growing well in

TABLE 1

Growth Responses and Synthesis of Vitamins by Organisms Isolated From Cheese

Organism	Source	Growth response	Vitamins synthesized		
			Riboflavin	Niacin	Biotin
			γ/gm.	γ/gm.	γ/gm.
Bacterium	Brie	Growth in presence of liver; no growth in synthetic media	....	....	......
Yeast	Brie	Growth in all media; some stimulation by liver	41	43	0.76
Bacterium	Brie	Growth in all media; some stimulation by liver	57	29	0.99
Yeast	Liederkranz	Growth in all media	69	91	0.89
Bacterium	Liederkranz	Growth in medium supplemented with liver	....	....	......
Bacterium	Liederkranz	Partial deficiency for nicotinic acid	97	....	0.22
Bacterium	Limburger	Good growth in all media	43	29	1.00
Yeast	Camembert	Deficiency for biotin	78	116	......
Penicillium camemberti	Camembert	Good growth in all media; stimulated by liver	81	118	2.46
Oidium lactis	University of Wisconsin	Good growth in all media	212	98	2.65
Bacterium linens	University of Wisconsin	Deficiency for pantothenic acid	....	....	......

media with or without added vitamins. Others show certain deficiencies. *Bacterium linens* showed a deficiency for pantothenic acid, a bacterium isolated from Liederkranz was deficient for nicotinic acid, and a yeast taken from Camembert required biotin. It seems fairly certain that in the mixed flora of cheese, some organisms are autotrophic for certain vitamins while others are heterotrophic for these compounds. Depending upon the metabolism of the dominant species of bacteria and fungi present, various fluctuations in vitamins might be expected to occur at different times in different kinds of cheese.

Further experiments were performed to determine the extent to which some of these microorganisms might synthesize and accumulate vitamins of the B complex in simple culture media as employed in earlier work on bacteria by Burkholder and McVeigh (1942) and on yeasts by Burkholder (1943). The procedure consisted of growing pure cultures in basal media in darkness and then assaying the entire cultures for vitamins according to techniques reported elsewhere by Burkholder and McVeigh (1942).

Results of the assays for riboflavin, nicotinic acid, and biotin produced by eight kinds of microorganisms cultivated for one week in a chemically defined medium are presented (Table 1). The values are expressed in micrograms per gram of fresh organisms present in the cultures at the end of the growth period, but it should be emphasized that the assays were performed on the organisms plus the culture medium in which they had grown. The data indicate that considerable quantities of certain vitamins accumulate in the organisms and in their environment following a period of rapid growth. The supernatant liquid in which *Oidium lactis* had grown acquired a distinct yellow color owing to the accumulation of riboflavin. These observations suggest that the cheese organisms which synthesize vitamins could contribute appreciable amounts of these substances to the cheese substrates upon which and in which they grow so luxuriantly.

In view of the reported difficulties encountered in microbiological assays for vitamins in dairy products it was considered necessary to perform some preliminary experiments with certain of the test organisms employed in the assays. The response of *Lactobacillus casei* to increasing dosages of cheese extracts added to basal test media were observed both in the presence and absence of riboflavin. In like manner, the responses of *L. arabinosus* were tested in basal medium containing increasing dosages of cheese extracts, both in the presence and in the absence of nicotinic acid. This part of the investigation was designed to test the extent to which other substances might interfere with the assay for riboflavin and niacin in complex extracts derived from cheese.

Extracts were prepared from the surface and core of Liederkranz and Camembert cheeses by grinding one-gram portions in 60 ml. of 1N H_2SO_4. The suspensions were autoclaved at 15 pounds pressure for 30 minutes, allowed to cool, the reaction adjusted to pH 4.7, and the volume made up to 100 ml. The extracts were filtered clear with the aid of Büchner funnels, No. 42 Whatman paper, and supercel. Different volumes of cheese extract were added to duplicate sets of culture tubes so as to supply increasing dosages equivalent to from one to 50 mg. of ripened cheese per tube.

Sufficient distilled water was added to the tubes where necessary to bring all volumes to five ml. Five ml. of basal medium were added to each tube to make a total of 10 ml. One series of tubes received only basal medium, while to another was added basal medium supplemented with 400 micrograms of riboflavin per liter (for the cultures of *L. casei*) or 300 micrograms of niacin per liter (for *L. arabinosus*). The plugged tubes of media were sterilized by autoclaving at 120°C.(248°F.) for 15 minutes. After inoculating each tube with a drop of pure bacterial sus-

pension of the required species, growth was allowed to take place at 37°C. (98.6°F.) for 72 hours. The acid produced during the growth period was titrated with .1 N NaOH.

DISCUSSION

The data (Fig. 1 and Table 2) show several interesting features. When extracts from either the surface or the core of Liederkranz and Limburger cheese were added to medium containing an excess of nicotinic acid, no effect was noted upon growth or acid production of *L. arabinosus*. This would indicate the presence of growth factors in adequate amounts already in the basal medium so that addition of cheese extract produced no greater activity of the bacteria.

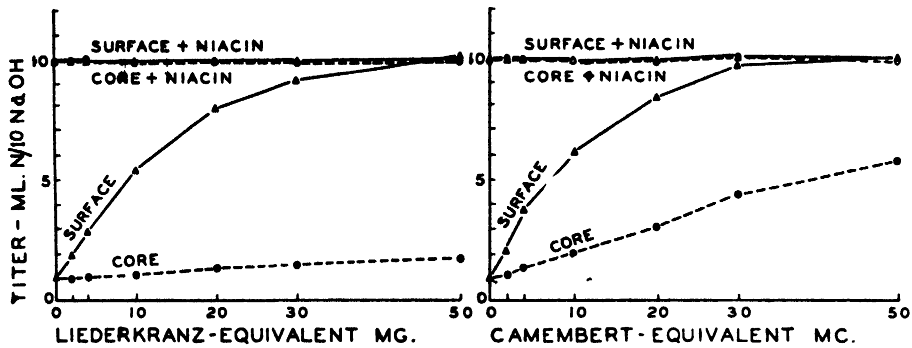

FIG. 1. Influence of extracts derived from Liederkranz and Camembert cheeses upon acid production of *Lactobacillus arabinosus* cultivated in medium of Snell and Wright (1941). With nicotinic acid present in the medium in the amount of 300 micrograms per liter, acid production was not altered by addition of extracts up to the equivalent of 50 mg. of cheese per 10 ml. of medium. Acid production in the basal medium appears to vary directly with the niacin present in increasing dosages of extract within limits.

Increased amounts of cheese extract added to basal medium containing no other nicotinic acid produced bacterial responses in proportion to the niacin content of the extracts within limits. The maximum acid production per tube was in all cases equivalent to approximately 9.9 ml. of .1N NaOH,

TABLE 2

Response of Lactobacillus casei[1] to Extracts of Cheese Added to Basal Medium With and Without Excess Vitamin B_2

Dosage of cheese extract in equivalent mg. per 10 c.c. culture		0	1	2	5	10	30
B_2 in medium	Sample	Nicotinic acid (ml.)					
No B_2	Liederkranz surface	0.3	0.8	1.1	1.8	2.8	9.1
No B_2	Liederkranz core	0.3	....	0.9	1.5	2.1	7.5
400 γ B_2/1	Liederkranz surface	8.8	8.8	8.8	8.9	8.9	8.9
400 γ B_2/1	Liederkranz core	8.8	8.6	8.7	8.9	8.9	9.0
No B_2	Camembert surface	0.3	0.9	1.1	1.7	2.6	7.9
No B_2	Camembert core	0.3	0.7	0.8	1.3	2.7	4.8
400 γ B_2/1	Camembert surface	8.8	8.7	8.8	8.8	8.9	9.0
400 γ B_2/1	Camembert core	8.8	8.6	8.8	8.9	9.0	9.0

[1] Period of growth was 72 hours at 37°C.(98.6°F.). Acid produced by cultures is expressed as milliliters of N/10 NaOH required to titrate each 10-ml. culture to pH 7.

whether the organisms were stimulated by the addition of excess niacin, niacin plus cheese extract, or only cheese extract. The growth-promoting effects of the surface samples of Liederkranz and Limburger cheeses greatly exceeded the effects of equivalent amounts from the cores of the same cheeses when supplied to *L. arabinosus* as the sole source of nicotinic acid.

The experiments with *L. casei* ϵ yielded essentially the same kind of data (Table 2). Acid production by *L. casei* was not influenced by the addition of cheese extracts to the basal medium containing excess riboflavin.

The four kinds of fancy cheese used in these investigations were sampled at four or five different stages in the curing process. Samples were obtained at the surface including the outer one-eighth to one-fourth inch of rind, at the subsurface immediately inside the rind, and at the core of each cheese. The cheeses representing progressive stages of ripening of a given kind were derived from the same batch of curd. The sampling times for each are listed (Tables 3 to 6).

Preparation of the material for testing was accomplished by thoroughly mixing a weighed sample of cheese in 1N H_2SO_4 or in potassium phosphate buffer at pH 7. Preparations to be used in assays for riboflavin, niacin, biotin, and pyridoxine were autoclaved in 1N H_2SO_4 at 120°C. for 30 minutes. After cooling, the reaction of the material was adjusted with NaOH to pH 4.7 and the volume made to some standard amount. Usually .5 gram of cheese was treated with 30 ml. of 1N H_2SO_4 and the final volume adjusted to 50 ml. before filtration. Clear solutions were obtained

TABLE 3

Vitamins[1] in Camembert Cheese at Progressive Stages of Curing

Age	Origin of sample	Thiamine	Riboflavin	Pyridoxine	Pantothenic acid	Biotin	Nicotinic acid
No. 1, 6 hours after dipping, salted	Surface	1.1	3.6	....	....	.040	0.8
	Core	1.2	4.8	....	....	.040	0.9
Duplicate cheese	Surface	1.5	4.2	....	....	.040	1.7
	Core	1.1	3.4	....	....	.032	0.8
No. 2, age 8 days	Surface	2.5	4.7	....	....	.096	13.0
	Core	0.5	3.9	....	....	.020	1.1
Duplicate cheese	Surface	2.7	4.9	....	....	.191	17.2
	Core	0.3	3.8	....	....	.020	1.1
No. 3, age 16 days	Surface	3.7	4.2	....	....	.082	13.0
	Core	0.5	4.0	....	....	.019	0.9
Duplicate cheese	Surface	4.3	4.1	....	....	.120	20.6
	Core	0.4	4.0	....	....	.019	0.9
No. 4, as normally consumed, age 44 days	Surface	6.2	7.5	3.2	11.3	.337	25.0
	Subsurface	0.3	5.2	1.5	0.5	.033	3.5
	Core	0.5	5.1	1.2	0.2	.019	3.1
Duplicate cheese	Surface	5.9	5.8	2.8	16.0	.353	18.0
	Subsurface	0.4	4.2	1.4	0.6	.024	3.0
	Core	0.5	5.3	1.4	0.6	.027	2.4

[1] The values in this table and in Tables 4, 5, and 6, given in micrograms per gram, are calculated as thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, biotin methyl ester, and nicotinic acid.

TABLE 4

Vitamins[1] in Liederkranz Cheese at Progressive Stages of Curing

Age	Origin of sample	Thiamine	Riboflavin	Pyridoxine	Pantothenic acid	Biotin	Nicotinic acid
No. 1, 6 hours after dipping, not salted	Surface	1.4	3.0	....	....	.046	1.0
	Core	1.1	4.2	....	....	.043	0.4
No. 2, 7 days after dipping, salted	Surface	3.1	5.1	....	....	.060	10.3
	Core	0.5	3.7	....	....	.025	0.5
No. 3, age 14 days	Surface	2.4	4.9	....	....	.056	9.8
	Core	0.5	3.8	....	....	.017	0.7
No. 4, age 23 days	Surface	4.5	8.3	....	....	.164	6.5
	Core	0.6	3.2	....	....	.036	0.6
No. 5, as normally consumed, age 44 days	Surface	4.9	11.0	1.3	12.2	.194	11.9
	Subsurface	2.0	7.9	0.8	6.9	.045	1.2
	Core	0.8	6.0	0.6	9.0	.041	0.5

[1] Values given in micrograms per gram.

TABLE 5

Vitamins[1] in Brie Cheese at Progressive Stages of Curing

Age	Origin of sample	Thiamine	Riboflavin	Pyridoxine	Pantothenic acid	Biotin	Nicotinic acid
No. 1, 6 hours after dipping, not salted	Surface	1.0	3.7	....	....	.144	0.9
	Core	1.0	3.3	....	....	.033	0.9
No. 2, 2 days after dipping, salted	Surface	0.9	2.8	....	....	.032	1.4
	Core	1.1	4.1	....	....	.028	1.0
No. 3, age 14 days	Surface	2.8	4.7	....	....	.059	4.3
	Core	0.6	2.9	....	....	.024	0.4
No. 4, age 28 days	Surface	5.9	10.8	....	....	.104	10.4
	Core	0.9	3.0	....	....	.029	0.6
No. 5, as normally consumed, age 49 days	Surface	3.0	12.2	12.8	63.0	.268	10.1
	Subsurface	0.7	4.4	10.8	17.0	.045	0.6
	Core	0.6	2.8	5.9	14.0	.045	0.5

[1] Values given in micrograms per gram.

TABLE 6

Vitamins[1] in Limburger Cheese at Progressive Stages of Curing

Age	Origin of sample	Thiamine	Riboflavin	Pyridoxine	Pantothenic acid	Biotin	Nicotinic acid
No. 1, 6 hours after dipping, not salted	Surface	1.2	2.5	....	....	.027	0.9
	Core	1.2	3.2	....	....	.027	0.9
No. 2, 2 days after dipping, salted	Surface	1.1	3.0	....	....	.041	1.0
	Core	0.8	3.9	....	....	.030	0.9
No. 3, age 10 days	Surface	3.4	6.8	....	....	.152	8.2
	Core	0.6	3.7	....	....	.018	0.5
No. 4, age 21 days	Surface	4.4	14.4	....	....	.150	12.5
	Core	1.2	4.8	....	....	.030	0.2
No. 5, as normally consumed, age 35 days	Surface	6.0	13.2	7.7	13.8	.633	8.8
	Subsurface	0.7	8.2	0.2	3.1	.023	0.5
	Core	0.8	5.6	0.2	3.0	.034	0.4

[1] Values given in micrograms per gram.

by filtering through a Büchner funnel with Whatman No. 42 paper and supercel. Samples to be used in tests for pantothenic acid were prepared by hydrolysis with pancreatin in neutral phosphate solution for 24 hours at 37°C., with subsequent heating for 10 minutes at 100°C. (212°F.), acidification to pH 4.7, adjustment of the volume, and filtration through filter paper and supercel. The method of filtration probably resulted in producing a sample freed to a considerable extent from interfering substances, according to Strong and Carpenter (1942).

Tests for riboflavin, nicotinic acid, pantothenic acid, and biotin were performed according to the methods described by Snell and Strong (1941), Snell and Wright (1941), Pennington, Snell, Mitchell, McMahan, and Williams (1941), and Snell, Eakin, and Williams (1941). The test media were supplemented with somewhat larger amounts of vitamins than called for in the original formulae. Pyridoxine was assayed with a special strain of *Saccharomyces oviformis*, following the general procedure outlined in a previous paper, Burkholder (1943). Thiamine activity was estimated with the *Phycomyces* method, Bonner and Erickson (1938), by adding small weighed quantities of finely ground cheese to the basal medium. Portions of the pancreatin digested samples were also tested for thiamine by the thiochrome method of Conner and Straub (1941) and yeast fermentation method of Schultz, Atkin, and Frey (1942). A few supplementary tests for riboflavin were performed fluorometrically according to the method of Ferrebee (1940).

The observed values at the surface and core of fresh curds and ripe cheeses are presented (Fig. 2) and results of the microbiological assays (Tables 3, 4, 5, and 6). These data as a whole indicate that relatively little change in vitamin content occurs in the central portions of fancy cheese cakes during the curing process. The superficial layers of the cheeses show apparent increases which tend to be very great in certain instances. The thiochrome determinations of thiamine in Camembert cheese indicated progressive increase in this vitamin during ripening, although the values throughout were considerably lower than those obtained with the biological method. The determinations of thiamine by the *Phycomyces* method are higher than those generally reported for cheese in the literature, Booher *et al.* (1942). In general the results with the physicochemical and fermentation methods were not considered reliable because of incomplete hydrolysis of the comparatively large amounts of protein employed in preparation of the samples for these tests (five gm. cheese in 30 ml. phosphate buffer). This statement is not to be taken as adverse criticism of these assay methods which are reliable when performed on properly prepared samples. If the microbiological data actually represent vitamin values, rather than artifacts resulting from nonspecific, growth-promoting compounds present in the cheese extracts, then the question naturally arises as to the source of the increase in vitamins.

It has been suggested that part of the apparent increase in vitamin content in the superficial layers of these cheeses may be accounted for by progressive dehydration during curing, accompanied by some outward migration of vitamins in the capillary flow of water from the main body of the cheese cake. In cheeses like Liederkranz and Brie, which possess a

moist surface, it seems unlikely that concentration by processes resulting from dehydration can account for such large increases as those which have been found for nicotinic acid and other vitamins.

The work of Sullivan, Bloom, and Jarmol (1943), on whole bricks of Limburger cheese sampled at six stages of curing, indicated increases in pantothenic acid, nicotinic acid, and biotin which were largely independent of the slight changes in moisture content of the cheese cakes. In Limburger and Camembert, which may have relatively dry rinds, it would seem reasonable to attribute to dehydration at least a part of the increase in vitamin

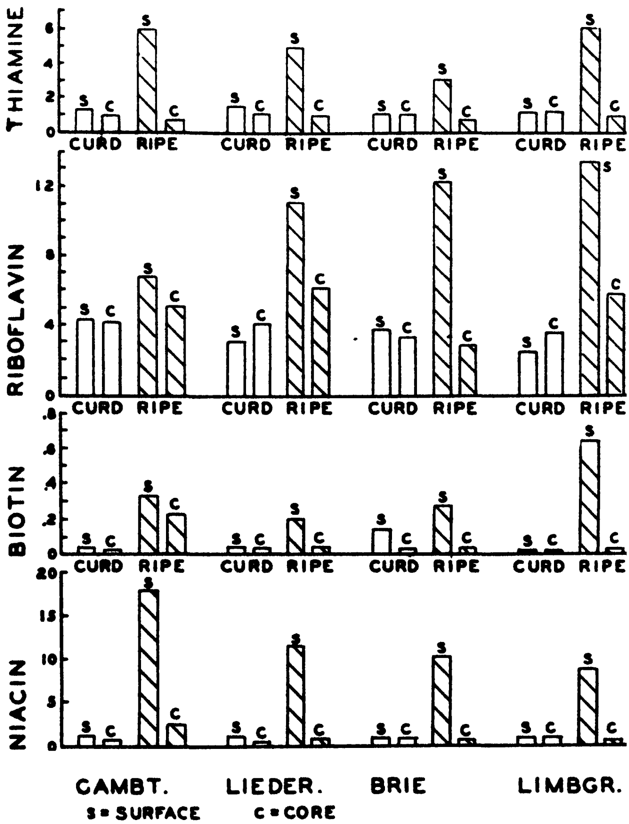

FIG. 2. Vitamin content of the surface and core of fresh curds and ripened cakes of Camembert, Liederkranz, Brie, and Limburger cheeses. Values are expressed as micrograms per gram, determined microbiologically. (See also Tables 3, 4, 5, and 6.)

content found in the superficial layers of the ripened cakes. The data contributed by the experiments reported in this paper and other supplementary information in the literature strongly suggest that production and storage of B vitamins are related to metabolic activities of microorganisms which grow luxuriantly in the exterior of certain kinds of fancy cheeses.

SUMMARY

Microorganisms isolated from the surface of four kinds of fancy cheeses synthesized considerable quantities of B vitamins in synthetic media.

Thiamine, riboflavin, biotin, and nicotinic acid, as determined microbiologically, appeared to increase in the surface layers of Camembert, Brie, Liederkranz, and Limburger cheeses through progressive stages of ripening. The evidence strongly suggests that vitamins of the B complex are synthesized by microorganisms and stored in the outer portions of certain fancy cheeses during the curing period.

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A QUANTITATIVE STUDY OF RATE OF DESTRUCTION OF AN *ACHROMOBACTER* SP. BY FREEZING[1]

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In connection with a study of the effect of fast-freezing upon the bacterial flora of mackerel, by Kiser and Beckwith (1942), it was decided to perform tests in which the effect of the freezing could be followed more closely and quantitatively in an attempt to determine whether the laws of disinfection, as described by Chick (1908, 1910), Phelps (1911), and Falk and Winslow (1926) for certain chemical agencies and heat, would operate likewise for freezing.

WORK OF PREVIOUS INVESTIGATORS

Some of the earlier experiments on the effect of freezing upon suspensions of bacteria were those of Park (1901) in which he froze suspensions of typhoid bacilli in distilled water at —5°C.(23°F.). Twenty-two weeks were required for sterilization of the suspensions. He also froze suspensions of typhoid and colon bacteria, *Staphylococcus*, and *Bacillus subtilis* in liquid air and determined that three per cent of the typhoid organisms, 5.5 per cent of the colon bacteria, 27 per cent of the *Staphylococci*, and 55 per cent of the *B. subtilis* were alive 130 minutes later.

Keith (1913) studied the factors influencing the survival of bacteria at low temperatures and was able to demonstrate that the presence of colloidal particles in a suspension, such as milk, exerts a protective influence, as does five per cent to 42 per cent of glycerol and 10 per cent of sucrose. He stated that a suspension of *Escherichia coli* was more than 99 per cent killed by —20°C.(—4°F.) in five days and was completely steriled in a few weeks, but that the formation of ice crystals constituted a mechanism important in killing the organisms. In 1915, Hilliard, Torossian, and Stone listed seven factors as involved in the germicidal effects of low temperatures: (1) the species or strain of bacteria, (2) the history of the strain, (3) the physical and chemical composition of the medium, (4) the temperature of freezing, (5) the duration of freezing, (6) the abruptness of temperature change, (7) the cultivation subsequent to thawing. However, Hilliard and Davis (1918) stated that the degree of cold below freezing was not important in the killing effect, but that repeated freezing and thawing were more lethal than freezing and holding at any temperature below 0°C.(32°F.). They shared the opinion of Keith that killing was largely due to the mechanical destruction of the microorganisms. Prucha and Brannon (1926) were able to demonstrate living typhoid bacilli in ice cream two years and four months after it had been frozen. Unfortunately their series of tests was interrupted at an important point

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so that their data cannot be graphed saisfactorily. Tanner and Williamson (1928), working mainly with yeast-cell suspensions, showed that the destruction of these organisms was proportional to the number of viable cells which remain at any given time.

Prescott (1931) reported a reduction in numbers of bacteria frozen in tap water in the ice trays of an ordinary refrigerator. Hess (1934) did a number of quantitative experiments on the effect of freezing upon suspensions of marine bacteria. He stated that the percentage of reduction of bacteria was increased by lower temperatures, longer exposure periods, rapid freezing, and repeated freezing. He also noted that a higher percentage of reduction occurred ordinarily in supercooled sea water than in frozen sea water. Old cultures were less sensitive to freezing than were young ones and organisms cultivated at —3°C.(26.6°F.) appeared to be more resistant than were those grown at 5 to 20°C.(41 to 68°F.)

EXPERIMENTAL PROCEDURE

The organism used was an actively proteolytic *Achromobacter* sp., chosen because it was very frequently encountered in the study of mackerel. The medium used was a sea-water broth, Kiser (1943). A 24-hour culture was diluted in sterile sea water and a number of tubes containing 10 ml. of a one-to-100 dilution of the original culture were prepared. These were frozen in an alcohol bath at —28°C.(—18.4°F.), and at suitable intervals duplicate tubes were removed and plated in duplicate upon a sea water-agar medium. The plates then were incubated for six days at room temperature before counting.

The results of these experiments (Table 1) show that there is an initial increase in numbers up to the 10th hour. This probably may be explained as due to the break-up of small clumps of bacteria by the freezing of the surrounding medium, as was suggested by Prucha and Brannon (1926). However, the possibility that the slight increase may have been due to multiplication during the brief time required to cool the medium to the freezing point should not be ruled out entirely. The organisms had ample nutriment, and it was demonstrated by ZoBell and Grant (1942) that growth of marine bacteria will occur in the presence of as little as one mg. of peptone per liter.

After the 10th hour the decrease in population is steady, and when the logarithms of the numbers of surviving bacteria are plotted against the time in hours, the points fall on a straight line during the first 300 hours. After this time, however, there is indicated a distinct decrease in the rate of death of the bacteria. Experiments extending over periods of 1,200 hours or longer show that in these suspensions the decrease in numbers is so much slower after the 300-hour point that sterility is not regularly complete. It is complete, however, in some instances.

If the equation of Arrhenius (1887) is applied to the figures which are derived from these experiments, it is found that a constant is obtained for the results of the first 300 hours, but that the values for the succeeding periods steadily decrease (Table 1). It will be recalled that the curves plotted from the data of Chick (1908, 1910) and of Falk and Winslow

(1926) showed similar characteristics. The latter authors, working with dilute solutions of $CaCl_2$ as a germicide, applied the equation for the specific reaction constants of reactions of higher orders to their data and concluded that during a portion of the entire period of observation the equation for the second order reaction yielded a satisfactory constant. In order to justify the application of this equation it would be necessary to postulate the presence of equimolecular amounts of the disinfectant and of the particular component of the bacterium affected by the disinfectant. It seemed feasible, therefore, to apply the equation to the results obtained in this research by freezing the organisms. The figures included herein do not show a constant which fits any period of observation.

TABLE 1

Quantitative Study of Death Rate of Achromobacter sp. by Freezing

Time	Organisms per ml	Log	$K_{\cdot}\times10^2$	$K_{\cdot}\times10^7$
0	8.2×10^5	5.91	......	....
2	7.7×10^5	5.89	......	....
5	8.98×10^5	5.95	......	....
7	9.22×10^5	5.96	......	....
10	9.9×10^5	6.00	.....	....
15	6.62×10^5	5.82	8.27	1.000
23	6.65×10^5	5.82	3.19	0.379
30	6.62×10^5	5.82	2.07	0.250
35	3.80×10^5	5.58	3.86	0.647
47	3.66×10^5	5.56	2.66	0.466
71	3.06×10^5	5.49	1.93	0.370
97	1.87×10^5	5.27	1.93	0.499
128	1.47×10^5	5.17	1.62	0.490
168	3.30×10^4	4.53	2.12	1.86
197	2.30×10^4	4.36	2.06	2.27
223	1.84×10^4	4.26	1.88	2.50
291	4.2×10^3	3.62	1.95	8.45
340	5.3×10^3	3.72	1.59	5.68
389	1.89×10^3	3.28	1.65	17.6
462	1.27×10^3	3.10	1.48	17.4
530	1.19×10^3	3.08	1.29	16.1
600	5.20×10^2	2.72	1.28	32.6
671	4.40×10^2	2.64	1.17	34.4

SUMMARY

It is shown that during the first 300 hours of freezing at —28°C. (—18.4°F.) the destruction of the *Achromobacter* sp. employed was proportional to the number of viable organisms present at any given time. During subsequent periods, up to 1,200 hours, no such relationship existed, and a total sterilization of all samples did not always develop.

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OBSERVATIONS ON GROWTH OF THERMOPHILIC ANAEROBES IN BREWER'S MEDIUM

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Owing to difficulties and inconveniences involved in cultivating anaerobic bacteria, they have received less attention than aerobes. Proper media in which they will grow have had to be developed as well as special mechanical devices to eliminate oxygen. Owing to these difficulties anaerobic bacteria may have been overlooked in many determinations, such as the causes of spoilage of canned foods. This paper reports results of an investigation of the possible use of Brewer's (1939, 1940) thioglycollate medium for examination of spoilage of preserved (canned) foods. This medium seems to have been well received in pathogenic bacteriology.

In order to overcome difficulties involved in previous anaerobic methods Brewer (1939, 1940) suggested that sodium thioglycollate be added to media to maintain anaerobic conditions for extended periods of time. The basal medium consisted of pork infusion, thiopeptone, sodium chloride, sodium thioglycollate, small per cent of agar, and methylene blue 1/500,000, as an indicator of oxidation-reduction potential. The advantages of this medium over other anaerobic media were clarity, addition of all ingredients at once, and maintenance of anaerobic conditions so that it was unnecessary to heat the medium after standing in order to obtain growth of anaerobes. Organisms tested by Brewer were the common anaerobic pathogens as well as several strains of anaerobic streptococci. All grew well in this medium. Since he did not investigate anaerobes causing spoilage in canned foods, it seemed desirable to do so.

Confirmatory evidence of the value of Brewer's medium in growing mesophilic anaerobes was reported by Marshall, Gunnison, and Luxen (1940). They were able to detect the presence of both aerobic and anaerobic contaminants in biological products even though these biologics were preserved with merthiolate. Dried spores of *Clostridium tetani* developed readily in thioglycollate medium with as low an inoculum as 10 spores per tube.

In the production of cells for antigenic analysis of *Clostridium oedematiens*, beef heart infusion broth plus .5 per cent glucose has generally been used. However, this medium, in order to give good results, required inoculation soon after autoclaving. McClung (1940a) found the addition of .1 per cent sodium thioglycollate and .1 per cent agar gave satisfactory growth even though the inoculation was delayed for several days. Thioglycollate medium was found to be of value in culturing other strains of pathogenic anaerobes. After testing several species of anaerobic bacteria in different media McClung (1940b) concluded that the pathogenic forms and *Clostridium sporogenes* yielded best growth in dehydrated thioglycollate medium of Brewer and liver infusion with particles. *Clostridium*

acetobutylicum, however, did not develop readily in dehydrated thioglycolate medium. This was probably the first suggestion that the medium might not be suitable for all anaerobes. Reed and Orr (1941) reported use of media containing sodium thioglycollate for cultivation of gas-gangrene anaerobes. For isolating and determining cultural characteristics of these anaerobes various media containing sodium thioglycollate were used successfully.

The use of thioglycollate medium alone or in conjunction with other media in routine diagnostic work is recommended by Reid and Bowditch (1942). This medium proved superior to others in producing positive cultures from blood and uterine specimens.

Recently, Stern (1942) compared the growth of *Clostridium thermosaccharolyticum* and *Clostridium sporogenes* in anaerobic media including Brewer's medium. All media used allowed good growth of *Clostridium sporogenes* inoculated with suspensions containing from six to 60,000 spores per milliliter. *Clostridium thermosaccharolyticum* grew well in liver infusion plus liver particles, but in Brewer's medium growth was observed only when the inoculum contained 6,500 or more spores per milliliter. Slightly better results were obtained when 1.5 per cent agar was added to Brewer's medium. Studies on the oxidation-reduction potentials of the media used revealed no significant differences.

EXPERIMENTAL PROCEDURE

The standard medium for thermophilic anaerobes has been liver infusion with liver particles. This medium is usually stratified with agar or some type of anaerobic seal. Its use has been described by Cameron (1936). During an investigation of canned foods, Brewer's medium was compared with liver infusion as a means of detecting presence of thermophilic anaerobes in cans which spoiled when incubated at 55°C.(131°F.). Brewer's medium used in the following experiments contained one per cent of dextrose, and liver infusion medium contained liver particles.

The first examination was made on 13 cans of spinach. All of them swelled at 55°C. They were opened aseptically and one-half ml. of the liquid portion was introduced into duplicate tubes of each media; the results are reported (Table 1).

TABLE 1

Comparison of Growth of Thermophilic Anaerobes From Spinach in Liver Infusion and Brewer's Media

Time in hours	Liver infusion medium		Brewer's medium	
	Positive	Negative	Positive	Negative
24	12	1	3	10
96	13	0	7	6

Though the samples were obviously spoiled by thermophilic anaerobes, only seven of them caused gas production in Brewer's medium. Liver infusion medium, on the other hand, after 96 hours' incubation showed positive results in all samples. Growth and gas production was more prompt and vigorous in the latter medium.

No attempt was made in the first experiment to determine the number of organisms or spores introduced into the two media. Since all were positive in liver infusion medium and only half of the samples produced gas in Brewer's medium, five more samples of spinach from swelled cans incubated at 55°C. were used in both media. Decimal dilutions were made of the liquor from the cans in order to vary the amount of inoculum in each tube. One ml. of liquor taken directly from the spoiled can, was introduced into the first tube in each series. The remaining tubes in the series were inoculated with one ml. of each decimal dilution. They were incubated at 55°C. for 144 hours; observations were made every 24 hours. In Table 2 only the results for Samples 1 and 3 are reported. Samples 2, 4, and 5 gave results identical with Sample 1.

TABLE 2

Effect of Size of Inoculum on Growth of Thermophilic Anaerobes in Liver Infusion and Brewer's Media

Sample	Time in hours	Brewer's medium (dilution of inoculum in ml.)					Liver infusion medium (dilution of inoculum in ml.)				
		1	1	01	001	0001	1	1	.01	001	0001
1............	24	—	—	—	—	—	+	+	—	—	—
	48	+[1]	—	—	—	—	+	+	+	+	+
	144	+	+	—	—	—	+	+	+	+	+
3............	24	—	—	—	—	—	+	—	—	—	—
	48	+	—	—	—	—	+	+	+	+	—
	144	+	—	—	—	—	+	+	+	+	—

[1]+ denotes gas production.

Results of this experiment differ slightly from those in the previous one in that all samples gave positive results in Brewer's medium. This is probably due to increased inoculum, one ml. being introduced in each case, whereas in the previous experiment only one-half ml. was used. The two samples reported in the above experiment differed in the number of thermophilic anaerobes present. Sample 1 contained more than 10,000 spores per milliliter as determined by liver infusion medium. In Brewer's medium, gas was produced only in dilutions up to 100, indicating much poorer growth. In Sample 3 fewer organisms were present as determined in liver infusion medium. The count was between 1,000 and 10,000 per milliliter. Brewer's medium gave positive results only when one ml. was introduced directly from the spoiled food. These results indicate that for the detection of thermophilic anaerobes, Brewer's medium requires a large inoculum to give gas production which was considered a positive result.

In order to obtain more quantitative results liver infusion-agar shake cultures were made at the time of inoculating the other media. Counts were made on the colonies developing in the shake cultures. Four samples of spinach were used in this test; results are shown (Table 3).

Two of the four samples gave lower counts in liver infusion-agar shake cultures than in liver infusion medium. This is due to the fact that anaerobes do not develop as readily in media containing 1.5 per cent as in liquid media and that the upper part of the shake culture is not anaerobic. Results in Brewer's are similar to those reported in the previous table.

Further experiments using cans of asparagus, peas, corn, and beans, all of which swelled at 55°C.(131°F.), were similar to those given (Table 2). In this work, 74 cans of spoiled foods were tested. Results similar to those reported above were obtained.

As a check on anaerobic conditions in Brewer's medium, a suspension of a thermophilic anaerobe isolated from starch containing about 110,000 spores per milliliter was used to inoculate liver infusion medium, Brewer's medium, and Brewer's medium under vaseline seal. Decimal dilutions were made of this suspension and added to the respective media with the following results:

Liver infusion medium—gas in all tubes including .00001 ml.
Brewer's medium—gas only in the 1-ml. tube.
Brewer's medium under seal—gas only in the 1-ml. tube.

The above experiment was repeated, using liquor from a can of spoiled corn as an inoculum. Identical results were secured. No added advantage was obtained by sealing Brewer's medium. This indicated that sufficient anaerobic conditions were obtained in the medium for development of thermophilic anaerobes.

TABLE 3

Comparison of Colony Count Obtained in Liver Infusion-Agar Shake Culture With Growth in Liver Infusion and Brewer's Media

Sample	Liver infusion-agar shake culture (count per ml.)	Brewer's medium (highest positive dilution)	Liver infusion medium (highest positive dilution)
1	19,000	1 ml.	1/1,000
2	13,000	1/100	1/10,000
3	50,000	1 ml.	1/10,000
4	500.000	1/100	1/1.000.000

Liver infusion with liver particles has always been the standard medium for determining the presence of thermophilic anaerobes in sugar and starch. To determine whether Brewer's medium could be substituted for liver infusion, three samples of sugar and six starch samples known to contain thermophilic anaerobes were examined. Tests were run by the standard technic of the National Canners Association. In conjunction with these tests rough quantitative determinations were run in which decimal dilutions were made of the sugar or starch suspensions prepared for the above analysis. One ml. of each of these dilutions was added to the appropriate media; results are shown (Table 4).

Using the regular method in which liver infusion is the standard medium, presence of considerable thermophilic anaerobe contamination was demonstrated in the sugar samples. With Brewer's medium, however, no contamination whatever was indicated. Only two of the six starch samples were shown to contain anaerobes. The number of positive tubes with Brewer's medium, however, did not agree with the number positive by liver infusion media. Starch sample No. 2 was revealed to be excessively contaminated when cultured in liver infusion medium, whereas when cul-

tured in Brewer's medium, only one of the six tubes was positive. This sample would be condemned on the basis of results in liver infusion medium but would pass the standard if cultivated in Brewer's medium.

When dilutions of the starch suspensions and sugar solutions were inoculated into the two media, Brewer's failed to show positive results with sugar solutions even though liver infusion medium indicated the

TABLE 4

Numbers of Thermophilic Anaerobes in Starch and Sugar as Determined by Liver Infusion Medium and Brewer's Medium

Sample	Standard N.C.A. technic				Highest positive dilution (organisms per gram)	
	Liver infusion medium		Brewer's medium		Liver infusion medium	Brewer's medium
	Pos.	Neg.	Pos.	Neg.		
Sugar 1	3	3	0	6	1/10	0
Sugar 2	4	2	0	6	1/10	0
Sugar 3	2	4	0	6	1 ml.	0
Starch 1	4	2	1	5	1/10	1 ml.
Starch 2	5	1	1	5	1/10	1 ml.
Starch 3	4	2	0	6	1/10	0
Starch 4	3	3	0	6	1 ml.	0
Starch 5	2	4	0	6	1 ml.	0
Starch 6	4	2	0	6	1/10	0

presence of at least 10 spores per gram of sugar. With starch, two samples were positive when one gram of starch was added directly to the tubes of Brewer's medium. Other samples with approximately the same thermophilic contamination failed to produce positive results in any of the tubes. Growth of thermophilic anaerobes as indicated by gas production was always observed to be slower in Brewer's medium.

TABLE 5

Comparison of Growth of Thermophilic Anaerobes in Liver Thioglycollate and Brewer's Medium

Inoculum	Approximate number of spores per ml. in original inoculum	Highest dilution giving positive gas production	
		Liver thioglycollate	Brewer's medium
Culture 46	10,000	1/10	1 ml.
Culture 49	100,000	1/1,000	1 ml.
Bean liquor	10,000	1/10	1 ml.

Since Brewer's medium was shown to be less satisfactory than liver infusion medium for the growth of thermophilic anaerobes, a few modifications of this medium were attempted. Liver thioglycollate medium was prepared by making an infusion of 250 grams of fresh pork liver in a liter of water. To this was added all the ingredients normally present in Brewer's medium except the pork infusion. The inoculum used in this experiment consisted of two pure cultures of thermophilic anaerobes (Nos. 46 and 49) isolated from spoiled home-canned spinach and liquor from spoiled beans. The approximate number of spores in each suspension was

determined by liver infusion medium previous to inoculation. Decimal dilutions of these suspensions were made and one ml. of each added to the two media. Results are reported in Table 5.

Liver thioglycollate medium gave better development of these organisms than Brewer's medium. The results, with this new medium were not as satisfactory however, as liver infusion medium itself; for example, Culture 49 in liver infusion showed gas production for 100,000 spores per milliliter. Liver infusion thioglycollate was positive only up to one to 1,000.

In repeating the above experiment the amount of liver infusion used for the liver thioglycollate was doubled. The medium consisted of infusion from 500 grams of liver per liter and the usual ingredients of Brewer's

TABLE 6

Comparison of Growth of Thermophilic Anaerobes in Liver Infusion Thioglycollate, Brewer's Medium Plus Liver Infusion, and Brewer's Medium

Inoculum	Approximate number organisms in original suspension	Highest dilution positive growth and gas production		
		Liver thioglycollate	Brewer's plus liver infusion	Brewer's medium
Bean liquor	100,000	1/100,000	1/100,000	1 ml.
Spinach liquor	100,000	1/100,000	1/100,000	1/1,000
Culture 46	100,000	1/100,000	1/100,000	1 ml.
Culture 46	10	1/10	1 ml.	0
Culture 49	10,000	1/100	1/10,000	1 ml.
Culture 4	100	1/100	1 ml.	0
Culture 22	100,000	1/100,000	1/10,000	1 ml.
Culture 37	100	1/100	1 ml.	0
Culture 79	100,000	1/100,000	1/100,000	1 ml.
Culture 99	1,000	10	0	0
Culture 119	100	1/100	1 ml.	1 ml.
N.C.A. culture	100.000	1/100.000	1/10.000	1 ml.

medium added to this heavier infusion. A special Brewer's medium was also prepared. It was made by weighing out the dry ingredients of dehydrated Brewer's medium, adding 350 ml. of liver infusion and 650 ml. of water to make the liter of medium. This gave a special Brewer's medium containing the usual ingredients plus liver infusion. Inoculum used in these media consisted of liquor from cans of spoiled beans and spinach, eight cultures of thermophilic anaerobes isolated from spoiled canned food, and a pure culture of a thermophilic anaerobe obtained from the National Canners Association. The approximate numbers of spores in the inoculum were determined as before in liver infusion medium. Decimal dilutions were made and inoculated into the various media in one-ml. amounts. The cultures were incubated at 55°C. for 120 hours; readings were made at 24-hour intervals.

While the results reported (Table 6) are somewhat more variable than previous ones, they clearly show the superiority of liver thioglycollate medium and Brewer's plus liver infusion over Brewer's original medium for the growth of thermophilic anaerobes. Four of the 12 inocula failed

to give growth in Brewer's, only one failed to grow in Brewer's medium plus liver infusion, while all grew in liver thioglycollate. Results with this latter medium checked closer with the approximate numbers of organisms as determined by liver infusion medium. In two cases results with liver thioglycollate medium failed to agree with this latter number. Brewer's medium plus liver infusion failed seven times, whereas Brewer's medium never gave numbers equal to those found by liver infusion. Gas production required longer time in all thioglycollate media than in liver infusion medium.

Since addition of liver infusion to Brewer's medium permitted an increased development of thermophilic anaerobes, the effect of liver particles was tested. In preparing this medium, one gram of dried extracted liver was added to each tube containing 10 ml. of Brewer's medium. An inoculum of approximately 100,000 spores per milliliter was prepared from a thermophilic anaerobe isolated from spoiled canned corn. Duplicate tubes of this special Brewer's medium were inoculated with one ml. of each decimal dilution of the inoculum. At the same time similar inoculations were made into regular Brewer's medium and liver infusion medium for comparison. In these tests liver infusion medium gave growth and gas production from one ml. of a one-to-100,000 dilution of the inoculum in 48 hours. Brewer's medium with liver particles was positive in a dilution of one to 10,000 after 120 hours. Regular Brewer's medium gave gas formation only from one ml. and one-to-10 dilution of the inoculum during a week's incubation. These results indicate a beneficial effect of liver particles in Brewer's medium. The effect is, however, not sufficient to make this medium as satisfactory as liver infusion medium. Growth and gas formation was retarded in both cases where thioglycollate was present.

During the early part of this work, attempts were made to isolate pure cultures of thermophilic anaerobes from as many sources as possible. When liquor from spoiled canned food was cultured in Brewer's medium attempts to isolate the organisms in liver infusion medium from Brewer's media resulted many times in failure, even though there had been evidence of growth. This suggested a study of the effect of continued growth of these organisms in this medium. The inoculum used was a culture of a thermophilic anaerobe obtained from the National Canners Association. Organisms were grown in liver infusion medium and a suspension containing approximately 100,000 spores per milliliter was prepared. One ml. of this inoculum was added to duplicate tubes of Brewer's medium and liver infusion medium. Tubes were incubated 72 hours at 55°C. (131°F.). Gas was obtained in both media at this time. At the end of this incubation period, one ml. was taken from Brewer's medium and reinoculated into fresh Brewer's medium, and one ml. from the liver infusion medium reinoculated into new liver infusion medium. Growth in liver infusion medium was as vigorous as in the first transfer. In Brewer's medium, growth was retarded until the fourth day of incubation. After the third transfer into Brewer's medium, no growth or gas production occurred after a week's incubation. An estimation of the numbers of organisms in liver infusion medium in the third transfer indicated approximately 10,000,000 organisms per milliliter. These results indicate

that the thermophilic anaerobe used in these tests was unable to maintain growth in Brewer's medium.

At the completion of this work two new fluid thioglycollate media were obtained. These media were prepared according to recommendations of the National Institute of Health to be used for tests of sterility in biologics. They differ from Brewer's medium in that neither contains a pork infusion base. Besides the usual ingredients, medium designated as No. 1, Bacto Fluid thioglycollate medium (Brewer's) contained beef infusion, the other, designated as No. 2, Bacto Fluid thioglycollate medium (Linden) contained proteose peptone and yeast extract. The growth of thermophilic anaerobes in these media was compared with Brewer's and liver infusion media.

Three cultures of thermophilic anaerobes were chosen for this work. The organisms from these cultures were inoculated into cans of whole-grain corn, incubated at 55°C. until spoilage occurred, and then stored at room temperature until used for this work. Liquor from the cans was used as the inoculum for the four media in the test. As in the previous work, one ml. of the liquor from the spoiled can was introduced into the first tube in each series, the remaining tubes being inoculated with one ml. of each decimal dilution prepared from the liquor. Tubes were incubated for a week at thermophilic temperatures before recording final readings. The following results were obtained: In the liver infusion medium containing liver particles, the liquor in dilution of one to 1,000,000 gave positive gas production, indicating the presence of at least 1,000,000 organisms per milliliter. In this test, thioglycollate media No. 1 and No. 2 and Brewer's medium gave growth and gas production only in the first tube of the series to which had been added one ml. of the liquor directly from the can. Repeating this experiment, results similar to those reported above were obtained with all media except fluid thioglycollate medium No. 2. This medium gave growth and gas production in one ml. directly from the can and in dilution one to 10. This is probably not enough different from the results of the other thioglycollate media to be significant.

In the preparation of liver infusion medium plus liver particles, the reaction of the medium is adjusted to pH 6.8 to 7. The final reaction of the thioglycollate media was pH 7.4 to 7.6. Since it seemed possible that pH might be an inhibiting factor in the development of these organisms, the three thioglycollate media were prepared with reaction adjusted to pH 6.8. Cultures used in the previous work were prepared and added to duplicate tubes of media, one set without adjustment, the other adjusted to pH 6.8. Liver infusion medium indicated the inoculum contained more than 10,000,000 organisms per milliliter.

The results obtained in this work indicated that a reaction of pH 6.8 was more favorable to the development of thermophilic anaerobes than the more alkaline reaction to which the thioglycollate media is adjusted. Liver infusion medium gave gas production in dilution of one to 10,000,000 of the original inoculum in 48 hours. In the three thioglycollate media with reaction of pH 7.6 only the original one-ml. inoculation gave positive results at the end of a week's incubation. The thioglycollate media adjusted to pH 6.8 give positive results as follows:

Fluid thioglycollate No. 1—one-to- 1,000 dilution, 4 days.
Fluid thioglycollate No. 2—one-to-10,000 dilution, 4 days.
Brewer's medium—one-to-1,000 dilution, 4 days.

The results obtained indicate that a neutral reaction is more favorable for the growth of thermophilic anaerobes than the alkaline reaction of pH 7.6. With this correction of reaction, however, the three thioglycollate media do not give results comparable to liver infusion medium either in rate of gas production or ability to initiate growth when small inocula are used.

DISCUSSION

Thioglycollate media have been generally accepted for isolation and growth of mesophilic anaerobes from wounds, biologics, and similar materials. Reed and Orr (1941) used thioglycollate successfully for gas-gangrene anaerobes from wounds. McClung reported successful growth of all mesophilic anaerobes he tested with the exception of *Clostridium acetobutylicum.* Stern compared liver infusion with liver particles and Brewer's medium using *Clostridium sporogenes* and *Clostridium thermosaccharolyticum*. He found Brewer's medium satisfactory for the mesophilic anaerobe but was unable to grow the latter organism, a thermophilic anaerobe, in this medium unless a heavy inoculum was used. Using a pure culture of this organism, from 6,500 to 65,000 spores were required to initiate growth in Brewer's medium, whereas liver infusion gave growth readily with 650 spores and in some cases when as few as six spores were added.

The results reported in this paper are in accord with those reported by Stern for *Clostridium thermosaccharolyticum*. Growth was obtained in thioglycollate media only when large inocula were used. The organisms died out quite rapidly in these media on repeated transfer in that medium.

In the modifications attempted in this work liver infusion thioglycollate seemed to give the most promising results. It is almost as good as liver infusion medium, although a longer incubation is required. It has several disadvantages as prepared in this laboratory. It has not been possible by methods used so far to obtain this medium without some precipitation on sterilization. Anaerobic conditions are not maintained as well in the liver thioglycollate medium as in Brewer's medium as indicated by methylene blue. Addition of liver infusion or liver particles to Brewer's medium increases the efficiency of this medium for growth of thermophilic anaerobes. This, however, makes a more complicated medium and addition of liver particles invalidates one of the purposes of the medium, namely a clear liquid medium.

It seems evident that thioglycollate medium lacks some material essential to the development of thermophilic anaerobes or contains some inhibitive substance. Liver infusion or liver particles seem to supply this needed material or counteract the action of this inhibiting material.

One factor which plays some part in the inhibition is the pH to which the thioglycollate media are adjusted. Better growth with gas production occurred in these media when the reaction was adjusted to pH 6.8. This is, however, not the only factor since the correction of pH did not produce results comparable to those obtained in liver infusion medium.

Brewer's medium cannot be used satisfactorily for the analysis of starch or sugar samples for thermophilic anaerobes. Though the number of samples of these two materials used in this work was limited, growth of thermophilic anaerobes from other material indicated this conclusion is valid.

For detection of thermophilic anaerobes in food spoilage, thioglycollate media was not as satisfactory as liver infusion plus liver particles unless large numbers of organisms were present in the spoiled material. With these media it is possible to miss entirely the organisms in canned foods if they are present in small numbers.

CONCLUSIONS

Thioglycollate media are not as satisfactory for the growth of thermophilic anaerobes as is liver infusion medium. Growth and gas production by these organisms is retarded in thioglycollate media, and unless the organisms are present in considerable numbers, no growth results.

In examining foods for spoilage by thermophilic anaerobes liver infusion medium is more satisfactory for detecting these organisms than thioglycollate media. Brewer's thioglycollate medium cannot be substituted for liver infusion medium in examination of starch or sugar samples for the presence of thermophilic anaerobes.

The addition of liver infusion or liver particles to Brewer's medium greatly enhanced the growth of thermophilic anaerobes. These organisms developed best when the media were adjusted to pH 6.8. However, none of these changes resulted in a medium as satisfactory as liver infusion medium.

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EFFECT OF METHOD OF THAWING UPON LOSSES, SHEAR, AND PRESS FLUID OF FROZEN BEEFSTEAKS AND PORK ROASTS [1]

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The effect of freezing upon the quality of meat has been the subject of a number of studies in the last few years. The best method of handling frozen meat after removal from the storage locker has been given considerably less attention.

Child and Paul (1937) and Paul and Child (1937) found that the palatability, total moisture, drip, and tenderness of cooked beef and pork were unaffected by freezing or by thawing at a temperature of 24 to 25°C. (75.2 to 77°F.) or in the oven at 175°C.(347°F.). The roasts thawed at 175°C. took less time to cook when thawing was counted separately but were in the oven about one and one-half times as long per pound as those thawed before being placed in the oven. Oven-thawed roasts had greater evaporation losses during thawing and higher total losses. The roasts thawed at 24 to 25°C. gained in weight during thawing, apparently owing to the absorption of atmospheric moisture, and yielded the greatest amount of press fluid. The unfrozen meat required the longest cooking period. Brady, Frei, and Hickman (1942) reported that the percentages of total weight losses in beef, pork, and lamb steaks cut .6 inch thick were greater when cooked thawed than when cooked frozen.

The purpose of the studies here reported was to determine the effect upon the shear, press fluid, and percentage of losses when frozen beefsteaks and frozen pork roasts were thawed at room temperature, refrigerator temperature, and oven temperature.

EXPERIMENTAL PROCEDURE

Thirty-three paired steaks, one of each pair from the left and the other from the right side, cut from the short loins of five different beef carcasses were cooked for the first part of this study. Three of the paired loins were from animals slaughtered by the Department of Animal Husbandry of the Kansas Agricultural Experiment Station and two loins were purchased directly from a packing company in Kansas City.

The steaks were cut two inches thick, boned, and only the longissimus dorsi muscle with its covering of fat was used. They were double-wrapped in freezer-locker paper, frozen at approximately —23.3°C.(—10°F.), and then stored at —17.8°C.(0°F.) until such time as they were to be thawed by one of the three methods previously mentioned.

For the second part of the study, 48 paired pork-loin roasts, obtained from experimental animals of known history, were supplied by the Depart-

[1] Contribution No. 113, Department of Home Economics.

ment of Animal Husbandry. Six roasts were cut from each loin. They were weighed, labeled, double-wrapped in freezer-locker paper, and again weighed and labeled. These roasts were held at —10°F. for a week, then were removed to a freezer at 0°F., and held at that temperature until they were to be thawed, at which time they were again weighed.

The steaks were thawed (a) by holding at room temperature for 15 hours, (b) in the refrigerator at approximately 3.3°C.(38°F.) for 23 hours, or (c) in a specially built experimental oven at 200°C.(392°F.). The pork roasts were thawed (a) by holding at room temperature for 15 hours, (b) in the refrigerator for 48 hours, or (c) in the oven at 176.7°C.(350°F.). In the case of the oven-thawed meat, no attempt was made to divide the time in the oven into thawing period and cooking period. The steaks were cooked at an oven temperature of 392°F. to an internal temperature of 65.6°C.(150°F.) and the roasts at 350°F. to an internal temperature of 82.2°C.(180°F.).

The following determinations were made on the cooked steaks and roasts: cooking time per pound; percentage of cooking losses; tenderness, as measured by the Warner-Bratzler modified shear apparatus; press fluid, obtained from a 40-gram sample by means of the Carver press; loss of weight of pressed sample and percentages of fat and serum in the press fluid.

In the study of pork roasts other determinations included were the following: change in weight during storage; change in weight during thawing and amount of drip [2] of those roasts thawed at room and at refrigerator temperature; percentage of cooking loss owing to evaporation and to drippings and percentage of total loss.

The cut to be thawed by any particular method was selected at random, although from each animal the same number of cuts were thawed by each method.

DISCUSSION OF RESULTS

The results obtained with the individual steaks varied but not as widely as did those obtained with the pork roasts. The steaks were probably somewhat more uniform in shape and size, for only one muscle and the covering of fat were used. When the results obtained by the different methods of thawing were averaged (Table 1) it was found that the lowest percentages of cooking losses were for meat thawed at refrigerator temperature, and the highest percentages of cooking losses and the greatest shear were for steaks thawed in the oven. However, when the losses owing to evaporation and drip during thawing at room temperature and at refrigerator temperature were added to the cooking losses, the percentage weight loss was approximately that lost during the cooking of the steaks thawed in the oven. The least press fluid was obtained from steaks thawed at room temperature and the most from steaks thawed in the oven.

The steaks placed in the oven while hard-frozen required about one and one-half times as long to reach a given internal temperature as those

[2] The change in weight during thawing did not include drip, as the meat was thawed while still wrapped so the drip was held in the package. The drip was determined after opening the package.

thawed at room temperature. This does not agree with Lowe's (1943) statement that the cooking time for frozen chops and steaks must be increased to at least two or four times longer than for unfrozen meat. She advocated a lower cooking temperature but did not specify how low.

Statistical analyses of the results for beef show that any differences obtained in press fluid and in percentage of cooking losses with different methods of thawing were well within the bounds of experimental variation. The effect of method of thawing on the shear was not sufficient on any one carcass to produce a clear difference; but when all steaks were considered, the shear for those thawed in the oven was enough greater to show that this method was the least desirable from the standpoint of tenderness as measured by shear.

A summary of the results obtained for the roasts from each pork carcass at the three thawing temperatures is given (Table 2). When the individual roasts were considered, there was a wide range in results owing perhaps in part to the size and shape of the roasts used. The roasts varied

TABLE 1

Summary of Data for Steaks Thawed at Three Different Temperatures

Method of thawing	Loss during cooking	Cooking time per pound	Shear	Press fluid
	pct.	*min*	*lb*	*c.c.*
Room temperature	21.36	35	13.1	11.9
Refrigerator temperature	20.30	39	13.1	12.9
Oven temperature	21.56	52	14.5	13.5

in weight from 389 to 791 grams, but the large and small roasts were distributed among the three lots. The range in percentage of total loss was from 20.42 to 43.07, both extremes being for roasts thawed at room temperature; the shear values were from a low of 5.6 pounds to a high of 21.8 pounds, and the press fluid varied from 2.5 to 11.9 c.c. When averages were taken for the three methods of thawing, the results show the roasts thawed at room temperature were the most tender, yielded the least press fluid, and had the lowest percentage of cooking losses but the highest percentage of total losses. The roasts thawed in the oven had the greatest shear and the greatest percentage of cooking losses. The roasts thawed in the refrigerator had the greatest amount of press fluid and the lowest percentage of total losses.

However, when the results were analyzed statistically by applying the F test of Snedecor (1940) it was shown that the method of thawing probably made no real difference in the percentage of cooking losses, the amount of press fluid, and the loss of weight when pressed. Although the difference in shear owing to the method of thawing was slight, it was consistent enough to show that thawing at room temperature was slightly superior as far as shear was concerned. On the other hand, when the percentage of total loss was considered, thawing at room temperature was shown to be least desirable.

The loss in weight of the pressed samples was in line with the amount of press fluid obtained, hence the results are not reported. Centrifuging

TABLE 2

Summary of Determinations on Pork Roasts for Each Carcass at the Three Thawing Temperatures

Carcass No.	Shear			Press fluid			Cooking loss			Evaporation in oven			Total loss		
	Room temp.	Refrig-erator	Oven—350°F.	Room temp.	Refrig-erator	Oven—350°F.	Room temp.	Refrig-erator	Oven—350°F.	Room temp.	Refrig-erator	Oven—350°F.	Room temp.	Refrig-erator	Oven—350°F.
	lb.	*lb.*	*lb.*	*ml.*	*ml.*	*ml.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
9	16.07	18.06	17.79	6.40	7.12	6.65	32.21	33.61	33.79	22.58	22.76	23.69	34.32	34.32	34.03
10	11.08	12.19	13.26	8.04	7.56	6.46	30.67	32.92	32.44	19.25	19.49	20.35	33.29	33.65	32.74
11	13.65	13.03	14.10	8.13	9.95	9.98	26.59	27.22	29.06	19.68	18.65	19.43	33.91	28.62	29.27
12	14.40	15.88	14.99	7.92	8.38	8.88	32.82	32.38	32.93	21.60	21.91	22.46	36.33	33.46	33.58
13	11.88	13.31	12.81	4.74	7.13	7.54	34.44	34.15	36.47	22.12	23.72	24.78	37.07	32.33	36.80
14	8.13	7.80	11.07	12.82	13.31	9.94	22.68	22.21	24.71	12.79	14.00	13.45	23.41	23.06	24.84
15	18.09	19.82	19.83	5.81	6.50	6.90	29.88	32.54	32.11	17.45	20.88	19.06	32.08	33.26	32.55
17	10.41	13.79	12.39	6.44	6.97	7.59	40.50	37.79	37.29	25.29	22.11	23.59	39.97	36.86	37.18
Average	12.96	14.23	14.53	7.54	8.36	7.99	31.22	31.60	32.35	20.09	20.44	20.85	33.80	31.93	32.62

of the press fluid separated the serum and fat, but the ratio between the two was so inconsistent that no relationship could be found between this and any other factor being considered.

The cooking time per pound for roasts thawed by any one method varied greatly, owing largely to the difference in size and shape of the roasts. The average cooking time was 81 minutes per pound for roasts thawed at room temperature, 87 minutes per pound for roasts thawed in the refrigerator, and 95 minutes per pound for roasts thawed in the oven. The differences in cooking time with the different methods of thawing pork roasts is much less in proportion to the total cooking time than for the steaks.

A linear relationship between the percentage cooking losses and the total cooking time for pork roasts thawed by all three methods is shown by the coefficient of correlation (Table 3). The correlation between per-

TABLE 3

Correlation Between Cooking Losses and Cooking Time and Between Press Fluid and Cooking Time for Pork Roasts

Correlation	Temperatures			Number of samples
	Refrigerator	Room	Oven	
Percentage cooking losses and total cooking time	.49 [1]	.42 [2]	.42 [2]	32
Percentage cooking losses and cooking time per pound	.51 [1]	.50 [1]	.17	32
Press fluid and total cooking time	—.51 [1]	—.52 [1]	—.58 [1]	32
Press fluid and cooking time per pound	—.60 [1]	—.19	—.31	32

[1] Highly significant. [2] Significant.

centage of cooking losses and cooking time per pound was highly significant for pork roasts thawed at refrigerator and room temperatures. Shrewsbury *et al.* (1942) also found a high correlation between percentage of cooking losses and total cooking time for pork roasts but failed to show a relationship between the cooking losses and the cooking time per pound. In the work here reported there was a rather high negative correlation between press fluid and the total cooking time for all three methods of thawing and between press fluid and cooking time per pound for pork roasts thawed at refrigerator temperature.

SUMMARY

Thirty-three paired beefsteaks and 48 paired pork roasts were studied to determine the effect of thawing at room temperature, at refrigeration temperature, and at oven temperature upon the shear, press fluid, and percentage losses of the cooked meat. The results indicate that thawing beefsteaks and pork roasts at room temperature, at refrigerator temperature, and in the oven gave similar results. However both steaks and roasts thawed at oven temperature were slightly less tender and required a longer cooking time than those thawed by the other two methods. Steaks and roasts thawed at room temperature yielded the least press fluid and the roasts thawed at room temperature had the highest percentage of total loss.

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ACTION OF A β-GLUCOSIDASE IN THE CURING OF VANILLA

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Mature vanilla beans have little or no aroma previous to being cured. The aromatic qualities of the beans develop as a result of changes which take place during the curing process. The nature of these changes has not been well understood, vanilla curing having been always an empirical process without scientific basis.

Gobley, in 1858, according to Chalot and Bernard (1920), was the first to isolate vanillin, the most widely known of the aromatic constituents of vanilla. Goris (1924) separated glucovanillin from the beans and by incubating it with commercial emulsin obtained vanillin. Philippe Miller, according to Chalot and Bernard, was the first to suggest that vanillin formation was a fermentation process Lecomte (1913) showed the presence of a hydrolytic enzyme and an oxidase in the beans. According to Lecomte's theory, however, the hydrolytic enzyme would break up coniferin, which he suggested was present in the beans, into coniferyl alcohol and glucose and then the coniferyl alcohol would be acted upon by the oxidase to form vanillin. However, the presence of coniferin in vanilla has never been confirmed. The presence of a peroxidase in vanilla beans was determined in this laboratory by Arana (1940). Later, Balls and Arana (1941a) found that vanillin was acted upon by vanilla peroxidase *in vitro*.

Further studies herein reported elucidate further the process of vanillin formation in vanilla beans and the chemical changes which take place during the curing process.

DISTRIBUTION OF GLUCOVANILLIN IN THE BEANS

The vanilla bean is composed essentially of a central portion containing the seeds and placental tissue surrounded by a fleshy part or ovary wall; uncured pods of *Vanilla fragrans* (Salisb.) Ames in longitudinal and cross sections are shown (Fig. 1).

Chalot and Bernard (1920) state that Linné believed vanillin was formed in the central seed portion of the beans, while M. F. Mérat and A. J. de Lens thought, on the contrary, that it was formed in the pulp that surrounds the seeds.

In order to check this point, measurements of the glucovanillin content were made by Gortner (1938), using a modification of Bourquelot's biological method of examining plants for glucosides. This method consists of the addition of emulsin to an extract of the plant and the determination of the reducing sugar content after a period of incubation, a change in content indicating the presence of β-glucosides and its magnitude giving a rough indication of the quantity. In this paper the vanillin content of the sample before and after incubation is taken as a measure of the quan-

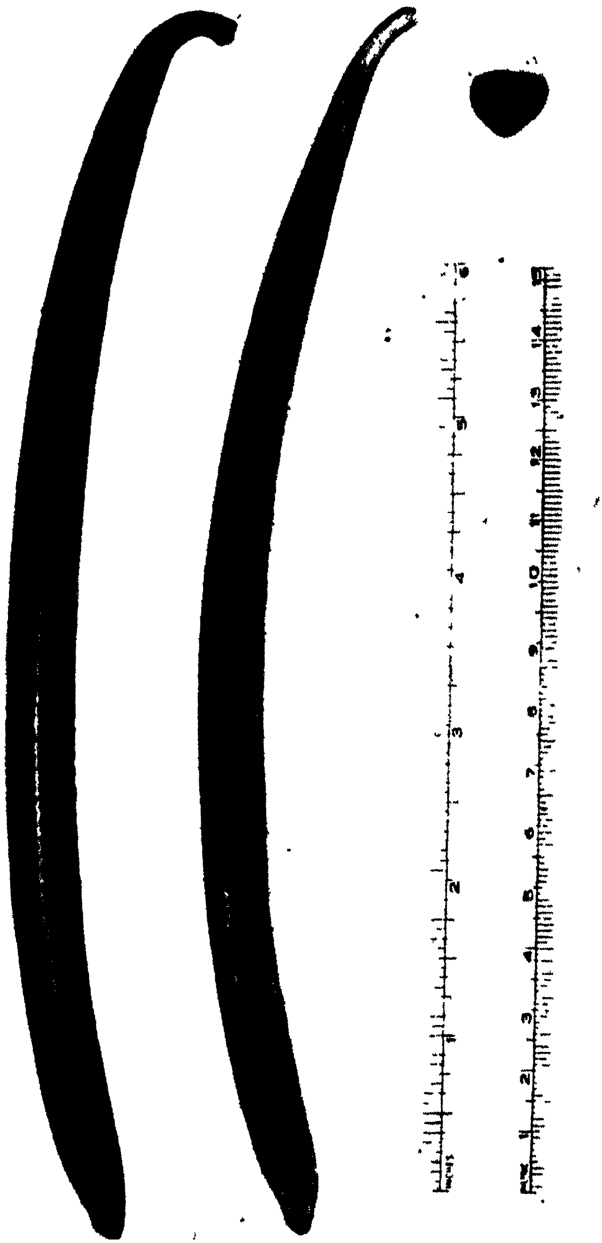

FIG. 1. Uncured beans of *Vanilla fragrans* (Salisb.) Ames showing the entire pod and longitudinal and cross sections. An active β-glucosidase which produces the hydrolysis of the vanillin-containing glucoside was found in the outer, fleshy portion or ovary wall. The glucoside was distributed throughout the outer portion and the central portion which includes seeds and placental tissue.

tity of glucovanillin that was present. Uncured vanilla beans seven to eight inches long were halved longitudinally and the central portion, including seeds and placental tissue, was separated from the outer fleshy portion. Each portion was stabilized and extracted by refluxing with 85-per cent alcohol plus a small amount of calcium carbonate to neutralize the acidity of the beans and thus prevent the hydrolysis of the glucoside. After boiling for one hour the liquid was removed, the residues were ground in an electric mixer with fresh alcohol and re-extracted for another hour. The extracts were combined, made to a definite volume, and the alcohol evaporated from aliquots, the volume being maintained by the addition of water. The extracts, to which several drops of toluene were added for preservation, were incubated with emulsin at 45°C.(113°F.) for seven days and the vanillin contents determined, according to the Association of Official Agricultural Chemists (1940). Duplicate determinations were made of each sample. Calculating the glucovanillin contents from the vanillin contents, showed 1.30 and 2.07 per cent glucovanillin in outer-portion samples of 90 and 85 per cent moisture, respectively, and 3.66 and 2.48 per cent glucovanillin in central-portion samples of 51.1 and 52.5 per cent moisture, respectively. Taking into account that the central portion averaged only 19 per cent of the weight of the fresh bean, it appears then that from .2 to .4 of the total glucovanillin of the bean was in the central portion.

In a similar manner the distribution of glucovanillin along the length of the vanilla bean was determined by analyzing samples of the blossom end, middle, and stem end. The results were, on the fresh basis, 1.01 and 1.76, .85 and 1.35, .64 and 1.00 per cent glucovanillin in the blossom-end, middle, and stem-end samples, respectively. These data show a definite gradient in glucoside concentration increasing from stem to blossom end. This partly accounts for the observed fact that vanillin crystals form during curing principally on the blossom end (Fig. 2). Taking the weight fractions of the beans into account along with the percentage shows that, of the total glucovanillin in the bean, about 40 per cent was in the blossom end, 40 per cent in the middle, and 20 per cent in the stem end.

EFFECT OF MATURITY OF VANILLA BEANS ON GLUCOVANILLIN AND VANILLIN CONTENT

Maturation of vanilla beans is indicated by a yellow coloration which develops in the blossom end of the pod. As ripening proceeds this coloration spreads along the entire bean and is usually accompanied by longitudinal splitting. The beans finally acquire a chocolate color.

In order to determine the effect of maturity on glucovanillin and vanillin contents, studies were made to determine the degree to which glucovanillin hydrolyzed during the natural ripening process. The vanillin and the reducing sugar contents, Association of Official Agricultural Chemists (1940), were determined in glucovanillin extracts of uncured beans eight to nine inches in length of different maturities before and after hydrolysis with emulsin (Table 1).

Vanillin was present only in traces in the whole green beans and in the split, blossom-end-yellow beans (Table 1), showing that there had

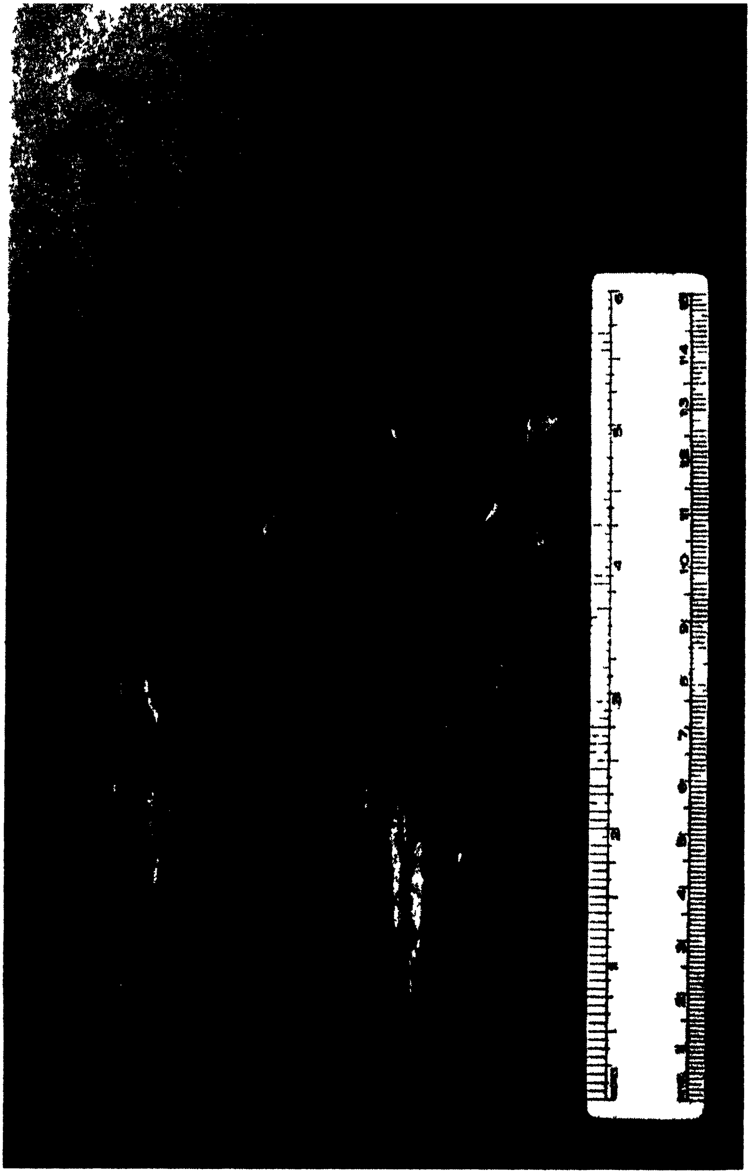

FIG 2 Cured vanilla beans showing the occurrence of vanillin crystals on the lower two thirds portion of the blossom end of the pods The glucovanillin was found to be present in largest quantities in the blossom end of the pod and decreased gradually toward the stem end

been only a slight hydrolysis of the glucovanillin up to the blossom-end-yellow stage of maturity. The vanillin content of the chocolate beans, however, was very high, showing that the glucoside was already hydrolyzed as a result of the natural ripening process. The total glucovanillin content of the treated glucoside extracts was about 8.8 per cent on a dry basis and apparently more was formed during the ripening from blossom-end-yellow to chocolate.

Calculating the glucovanillin content from the increase of reducing sugars upon hydrolysis showed glucovanillin contents of only about .5 per cent less for the first two degrees of maturity. The increase in

TABLE 1

Analyses of Glucoside Extracts From Uncured Vanilla Pods of Different Maturity Before and After Hydrolysis With Emulsin [1]

Maturity of beans	Moisture content	Analyses of extracts on dry basis				
		Reducing sugars		Vanillin content		Total gluco-vanillin content [2]
		Untreated	Treated	Untreated	Treated	
	pct.	*pct.*	*pct.*	*pct.*	*pct.*	*pct.*
Whole beans, entirely green......	80.6	2.68	7.65	0.10	4.23	8.74
Split beans, blossom end yellow	76.6	3.25	7.89	0.06	4.26	8.80
Chocolate-colored beans............	75.0	15.88	15.89	4.59 [3]	......	......

[1] Hydrolyses resulted from the incubation of glucovanillin extracts with commercial emlusin at 45°C. (113°F.) for five days. [2] As calculated from the vanillin content of the treated beans. [3] Corresponding to a glucovanillin content of 9.48 per cent.

reducing sugars and other substances giving a similar reaction with Fehling's solution from the yellow to the chocolate stage was greater than could be accounted for on the basis of glucovanillin hydrolysis alone.

β-GLUCOSIDASE OF VANILLA BEANS

Additional work on the glucovanillin-glucosidase system was directed along the line of studying the crude enzyme itself by observing the effects of various crude enzyme preparations on glucovanillin substrate prepared as described above. The hydrolysis of glucovanillin was followed by measuring the phenol value, Balls and Arana (1941b).

To show the presence and action of a β-glucosidase in the vanilla bean a crude enzyme was prepared by comminuting blossom-end-yellow beans (one part dry to 50 parts extract) in an electric blendor for five minutes, filtering, and incubating aliquots of the filtrate with glucovanillin extracts at 45°C. and the optimum β-glucosidase pH of 4.2 to 4.4, according to Waksman and Davison (1926). After six days the solutions were made to definite volume, filtered, and the total phenol value measured. Controls were effected by incubating aliquots of glucoside solution without enzyme and with enzyme extracts previously boiled for 20 minutes. Corrections were made also for the phenol value of the enzyme extracts when incubated alone. All of the subsequent experiments with crude enzyme preparations reported were carried out in the above manner.

The phenol values in per cent, dry basis, were 1.5 for glucovanillin incubated alone, 1.4 incubated with boiled enzyme, and 8.7 incubated with

the active enzyme. These data prove that the crude enzyme extracts from the vanilla beans brought about the hydrolysis of the glucoside. The fact that the phenol value of glucovanillin incubated alone was approximately the same as that of glucovanillin incubated with the boiled vanilla enzyme shows that the boiled enzyme solution did not act upon the glucoside solution. The hydrolysis was, therefore, enzymatic in character and the presence of a β-glucosidase in mature, blossom-end-yellow pods of vanilla is thereby confirmed.

Separation of Crude Enzyme: The β-glucosidase was separated from uncured, mature vanilla pods by a modification of the process used for the extraction of emulsin from sweet almonds, Waksman and Davison (1926). Four hundred grams of beans were comminuted for five minutes in an electric mixer with 800 milliliters of water. After standing one hour, the liquid was filtered through glass wool and the protein impurities precipitated by adding 60 drops of acetic acid. Since the mixture was

TABLE 2

Activity of a Preparation of β-Glucosidase on Glucovanillin

Treatment[1]	Phenol value, dry basis, after incubation	
	Sample 1[1]	Sample 2[1]
	pct.	*pct.*
Glucovanillin incubated with vanilla enzyme	2.15 [2]	3.02 [2]
Glucovanillin incubated with vanilla enzyme boiled for 20 minutes	1.55 [2]	2.07 [2]
Glucovanillin incubated alone	1.20	1.46

[1] Portions of β-glucosidase from 10 grams of beans were incubated at a pH of 4.3 at 45°C. (113°F.), with glucovanillin extracts from one gram of beans in Sample 1 and 1.2 grams in Sample 2. (All quantities on a dry basis.) [2] Since the phenol values of the fresh and boiled enzyme solutions incubated alone were zero, no corrections had to be made.

colloidal in character and could not be filtered by ordinary means, a Chamberland-Pasteur pressure filter was used. The crude enzyme was precipitated by adding to the filtrate four volumes of 95-per cent alcohol. The brownish powder obtained was dissolved in 160 milliliters of water and the activity tested by incubating 20-milliliter aliquots at a pH of 4.3, at 45°C.(113°F.), with aliquots of glucovanillin extracts, and measuring the phenol value after five days. Controls were carried out by incubating the glucoside with the enzyme solution boiled for 20 minutes; results are shown (Table 2).

Glucovanillin was hydrolyzed by the β-glucosidase separated from the uncured vanilla pods (Table 2). The corrected phenol value of the treated glucoside, owing to the action of the enzyme solution alone, was .95 per cent in one case and 1.56 per cent in the other. The activity of the enzyme, although not very high, was nevertheless definite.

Distribution of Enzyme: The distribution of the β-glucosidase in the vanilla pod was measured by incubating glucovanillin extracts with crude enzyme extracts of the outer fleshy portion of the beans and of the central portion, including seeds and placental tissue.

Corrected phenol values in per cent dry basis were 7.65 for the outer portion, 1.24 for the central portion, and 1.29 for glucovanillin incubated alone. Therefore it can be concluded that there was no active β-glucosidase

in the central seed portion and placental tissue of the beans, and all of the enzyme was in the fleshy part or thick wall of the pods.

Since it was shown that some glucovanillin exists in the central portion of the bean where there is no enzyme, it must be concluded that during curing this glucovanillin diffuses outward with the water and is hydrolyzed upon reaching the outer wall where the enzyme is located or the enzyme diffuses throughout the whole pod.

Effect of Maturity on Enzyme Activity: During the course of these investigations a large variation in glucosidase activity was found among beans at various stages of maturity. The effect of maturity on the enzyme activity was followed by incubating enzyme extracts of whole beans entirely green, whole beans with blossom ends yellow, and split beans with blossom ends yellow with glucovanillin. All the beans used were picked from the same vine and tested two days later.

The corrected phenol values in per cent, dry basis, were .12 for whole green beans; 4.30 for whole beans, blossom-end-yellow; and 6.95 for split beans, blossom-end-yellow. These figures show that while a negligible amount of active β-glucosidase was present in the green beans, more was present in blossom-end-yellow beans and the highest activity was found in split, blossom-end-yellow beans.

In a similar experiment green beans from the same vine were allowed to mature in the laboratory. Tests for activity made at intervals showed the following indices on the basis of phenol values: after harvest, one day, green, 0; four days, green, 3.9; 10 days, partly yellow, 7.9; and 14 days, third to half chocolate, 1.85. The green beans were devoid of activity; as coloring proceeded, in the yellow beans, the activity increased. Storage of the harvested green beans beyond about 10 days resulted in partial inactivation of the enzyme.

Reactivation of Enzyme: Beans in which the enzyme activity had receded to a low level were cured to ascertain whether the glucosidase had been permanently inactivated.

Curing consists usually of an initial killing treatment to stop the vegetative processes of the beans followed by sweating or slow drying in the sun or in an oven until beans become flexible. The beans are further dried at room temperature to the desired loss in weight and finally conditioned in boxes for at least three months.

The beans used in this experiment which had remained in the laboratory 14 days after harvest, were frozen for 16 hours, thawed six hours, and sweated at 45°C., according to work of Balls, Kevorkian, and Arana (1942). The phenol value before freezing, 1.85 per cent, dry basis, decreased to 1.55 after thawing but then increased to 5.05 after 24 hours' sweating. After 72 hours' sweating the index had receded to 2.45. Thus it is shown that by the application of heat the enzyme system can be rejuvenated to a level comparable with original activity.

Change of Enzyme Activity in Green Beans During Curing: An experiment was designed to measure the enzyme activity during curing of entirely green beans one day after harvest. One lot of beans was killed by a modification of the Bourbon method used in Réunion and Madagascar, which consisted of immersing the beans three times for 10 seconds at 30-second

intervals in water at 80°C.(176°F.), Chalot and Bernard (1920), while a second lot was killed by freezing for four hours followed by thawing for one hour at room temperature. Both lots of beans were then sweated in an electric oven at 45°C., for four days. Crude enzyme extracts of samples of the two lots were made at intervals and the activity on glucovanillin measured by determining the phenol value; results are shown (Table 3).

The glucosidase activity was very low throughout the curing process (Table 3); also, in these green beans the enzyme was not greatly activated by exposure to a temperature of 45°C. Curing of green beans usually results in an inferior product and the above experiment indicates that the poor results are due to the low-activity level of the enzyme system.

TABLE 3

Activity of β-Glucosidase in Entirely Green Vanilla Beans Subjected to Different Curing Processes, 24 Hours After Harvest[1]

Killing process	Phenol value (dry basis)[2]			
	Fresh beans	One hour after killing	After 24 hours' sweating	After 96 hours' sweating
	pct.	*pct.*	*pct.*	*pct.*
Hot water	.30	.65	.30	.55
Freezing	.30	.95	.50	.85

[1] Glucovanillin extracts from .5 gram of beans were treated with enzyme extracts from .3 gram of beans, both quantities on a dry basis, for five days at a pH of 4.2 to 4.4 at 45°C.(113°F.).
[2] Corrections were made for the phenol value of glucoside and enzyme extracts incubated alone.

On the other hand, the fact that such beans are of passable quality confirms previous observations by Balls and Arana (1941a) that the quality of vanilla beans is not entirely due to the presence of vanillin.

DISCUSSION

In the light of what is now known, the process of curing vanilla beans can be elucidated in some further detail than was possible previously. The bean can be regarded as a complex chemical system consisting, in part, of vanillin and a β-glucosidase which reacts to form vanillin and glucose. From considerations of quality as well as from analytical criteria, it is quite certain that the glucovanillin-vanillin transformation is not the only important one that occurs during curing, Balls and Arana (1941a). From lack of knowledge of the other changes, however, principal consideration can now be given only to the vanillin-forming reaction.

In the bean itself most of the glucovanillin is present in the fleshy part or ovary wall where the β-glucosidase that catalyzes the hydrolysis of the glucoside is also located. In regard to the extent of hydrolysis of the uncured bean at various stages of maturity, it is known that in mature beans the vanillin exists almost entirely as the glucoside. In approaching the chocolate stage more glucovanillin is formed, and all of it proceeds to vanillin.

In a parallel manner the activity of the enzyme starts at zero in the green stage and increases with maturity. In the chocolate bean it apparently decreases. The activity of the remainder of the enzyme system during maturation is not known.

Curing the green beans, which has been observed to result in a product of low vanillin content, is therefore due to the lack of β-glucosidase activity, but the fact that the product is of passable quality indicates that changes other than that of glucovanillin to vanillin are important in the curing. Curing blossom-end-yellow beans in which the enzyme activity is appreciable results in an excellent product with high vanillin content.

During the first stages of the curing process glucovanillin is hydrolyzed to vanillin. The further changes that contribute to quality take place very slowly during the conditioning stage.

It is of interest to note that beans can be aged before curing to allow their enzyme activity to increase far above that of the green beans and that if beans have passed the high activity stage, they can be heated to rejuvenate the enzyme system to a certain extent.

SUMMARY

The distribution of glucovanillin and of a β-glucosidase in the vanilla bean and the effect of maturity on the amount of glucovanillin and on glucosidase activity have been determined. A crude preparation of the β-glucosidase has been made and its activity followed in the beans throughout the first stages of the curing process. Reactivation of the partially inactivated enzyme has been effected by the application of heat.

ACKNOWLEDGMENT

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EDITORIAL REVIEW

HOME PRACTICES AND THE NUTRITIVE VALUE OF FRUITS AND VEGETABLES

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The food industry is taking pains to market foods which are high in nutritive value, palatable, and appropriately packaged, but too little attention has been paid until recently to the effect of home practices on food quality. (The present national food problems, however, have made it imperative that the greatest possible conservation of nutritive value be achieved; and the important part which home handling of food plays in attaining this end is becoming recognized.)

As a stimulus to better understanding of the importance of home care of food and to further research in this field, this review is presented. It is confined to a consideration of some of the information concerning the nutritive value of fruits and vegetables. Even this limited field has been covered by no means completely, but the extent to which home care matters is indicated, and some of the instances where further research is needed are pointed out.

SELECTION OF FRUITS AND VEGETABLES

Considerable evidence has accumulated to indicate that there is wide variability in the nutritive value of fresh fruits and vegetables, according to their variety and stage of maturity and also according to growing conditions, such as soil and climate. Much of this information is of special interest to growers. Some of the data, however, are valuable aids in the selection of fruits and vegetables from the market. Some of these will be noted.

Tressler, Mack, and Jenkins (1937) found that when lima beans of a single variety were graded for size, the small beans contained much more ascorbic acid on a percentage basis than did the large ones. Small, mature peas have been found by Todhunter and Sparling (1938) to be higher in ascorbic acid per unit weight than larger, mature ones of the same variety; and Mack and Tressler (1936) found that in a given variety of peas the ascorbic acid was inversely proportional to their sieve size. Similarly, a study by Murphy (1941) showed that small onions contain from 32 to 141 per cent more ascorbic acid than large onions of the same variety. Findings with regard to the relationship between the size of tomatoes and their ascorbic acid content are not consistent. One study by Brown and Moser (1941) supports the theory of an inverse relationship between the ascorbic acid content of the fruit and its size, but another by Tripp and Satterfield (1937) failed to find any such consistent relationship. When large and small, mature potatoes were examined by Esselen, Lyons, and Fellers

(1942), no correlation was found between the ascorbic acid content and the size of the tubers.

The stage of maturity seems to be important in determining vitamin content in many instances, but by no means always. Woods (1935) found that new, immature potatoes contained twice as much ascorbic acid as did mature ones. Leverton (1937) found that while the differences in the ascorbic acid value between green and ripe bananas were not great, the ripe samples were always higher in this vitamin than were green ones taken earlier from the same hands. Harris and Poland (1939) found that bananas increased during ripening to the maximum stage of ripeness at which the fruit is usually eaten, and then decreased as the fruit became overripe.

Olliver (1938) found that the average concentration of ascorbic acid in gooseberries and black currants rose in the initial stages of development and then fell as the season progressed. The ascorbic acid concentration of strawberries, however, fell during the early stages of development and then rose as the fruit changed color. Subsequently, the average value for strawberries fell again, so that samples of the later crop were not so rich in ascorbic acid as were those of the early crop. Mack and Tressler (1936) found that as peas matured the percentage of ascorbic acid decreased. Tripp and Satterfield (1937) found that soft and overripe tomatoes were lower in ascorbic acid value than firm, ripe ones. On the other hand, Bracewell, Hoyle, and Zilva (1930), in studying apples, found that Bramley's seedlings (high in ascorbic acid), when picked from the tree two weeks before harvesting the normal crop, had approximately the same ascorbic acid as those of the normal crop. Spinach was shown by Tressler, Mack, and King (1936) not to change materially in the harvesting period of one week. Green and ripe grapes (Scuppernong variety) have been found by Bell, Yarbrough, Clegg, and Satterfield (1942) to contain about the same amount of ascorbic acid; and the change in the percentage of this nutrient in the maturing of citrus fruits, as determined by French and Abbott (1940), was not large.

Considerable evidence has been obtained to suggest that greenness in plant tissue is indicative of a high content of certain nutrients. Dye, Medlock, and Crist (1927) found that the outside green leaves of head lettuce were far superior in vitamin A to the inside yellow ones and that leaf lettuce was superior to head lettuce in this respect. Confirming this, Kramer, Boehm, and Williams (1929) found that the dark green outer leaves of head lettuce contained 30 or more times as much vitamin A as the whitest leaves from the centers of the same heads. Similarly, Roscoe (1930) found that the riboflavin content was higher in green lettuce leaves than in pale ones. In this study, no such difference was found, however, in the distribution of thiamin.

HOME STORAGE

Prolonged storage of apples has been shown by Manville, McMinis, and Chuinard (1936) to cause more destruction of ascorbic acid than of vitamin A, and the ascorbic acid of the Jonathan variety was found to be less stable than that of the Delicious. Peas in the pod held at room temperature were found by Mack and Tressler (1936) to lose their ascorbic acid rather

rapidly, but refrigeration reduced the loss considerably. Lima beans were held in their pods by Tressler, Mack, and Jenkins (1937) after harvesting and were found to lose their ascorbic acid slowly, but when shelled the beans lost this vitamin about twice as rapidly. Refrigeration was found to retard such loss appreciably. Spinach, when held at room temperature, was found by Tressler, Mack, and King (1936) to lose half of its ascorbic acid in three days and practically all of it in seven days, but when stored at 1 to 3°C.(33.8 to 37.4°F.), it lost this vitamin very slowly. Wheeler, Tressler, and King (1939) held broccoli, cauliflower, endive, lettuce, kale, and New Zealand spinach at room temperature in the summer and found that these vegetables lost their ascorbic acid rapidly, but the losses from broccoli and cauliflower were reduced to an almost negligible figure by refrigeration. In comparison with other vegetables, snap beans have been found by Mack, Tapley, and King (1939) to lose their ascorbic acid rapidly at all temperatures, but the rate of loss was somewhat less at lower temperatures.

Five months of common storage of potatoes at 4.4 to 10°C.(40 to 50°F.) has been shown by Esselen, Lyons, and Fellers (1942) to cause losses of 28 to 63 per cent of their ascorbic acid in seven varieties, but in an eighth variety the loss was only 2.5 per cent. Woods (1935), however, found that common storage of the mature potato from three to eight months did not change the ascorbic acid content to any marked degree. Since one of the recommended methods for onion storage is holding them in the attic, this procedure was studied by Murphy (1941) with reference to its effect on ascorbic acid. It was found that these conditions cause rather serious losses of ascorbic acid even in relatively short periods. Mayfield and Richardson (1940) found that parsnips, allowed to stand in the frozen ground during the winter, lost by spring 60 to 70 per cent of the ascorbic acid value which they had in the fall when mature and ready for harvest. In one study by Brown and Moser (1941), tomatoes held for 18 days at laboratory and refrigerator temperatures lost no ascorbic acid.

The advantage of using a refrigerator hydrator has been demonstrated in a study by Harris and Mosher (1941) of its effect on the keeping quality of lettuce. The rate of destruction of provitamin A was found to be apparently linear until about 40 per cent of its weight was lost, then the destruction was accelerated. Since the lettuce was considered wilted when it had lost 30 per cent of its moisture, wilting was suggested as an index of loss of vitamin A.

PREPARATION PRELIMINARY TO COOKING

There is always a question as to whether it matters if certain portions of fruits and vegetables are discarded in the course of their preparation. Answers to a few of these problems have been obtained.

Several investigators have reported that the skin of the apple is a better source of ascorbic acid than the inner portion. Bracewell, Kidd, West, and Zilva (1931) reported that the concentration of ascorbic acid in apple tissue increased as the skin was approached from the core and was more than six times as great in the flesh near the peel as it was in the flesh near the core. A more recent publication by Manville, McMinis,

and Chuinard (1936), however, has pointed out that careful removal of the skin does not decrease markedly the ascorbic acid from the apple.

The skins of grapes (scuppernong variety) have been shown by Bell, Yarbrough, Clegg, and Satterfield (1942) to contain three times as much ascorbic acid as the inner portion of the fruit. Pfund and Nutting (1942), in studying potatoes, have shown that while the outer portion of this vegetable contains a higher concentration of iron than the inner portion, this high concentration is probably not immediately beneath the skin. The same authors showed that the thick, crusty skins of baked potatoes are high in iron.

Much interest has been expressed as to whether the stems of various greens may be discarded without serious sacrifice of nutrients. Sheets, Leonard, and Gieger (1941) found that the leaves of collards, mustard, lamb's quarters, and pokeweed contained about twice as much iron as the petioles and two or three times as much iron as the petioles and stalks combined. The leaf blades of mustard and turnip tops contained from three to four times as much iron as their petioles and midribs.

Similarly, Barnes, Tressler, and Fenton (1943) found that in broccoli the upper three-fourths inch of stalk contained more ascorbic acid than did the lower three-fourths inch. The top half of rhubarb stalk has been found by Brown, Schuele, and Fenton (1941) to contain almost twice as much ascorbic acid as the lower half and the top four inches almost three times as much. The central leaves of onion bulb were found by Murphy (1941) to be much higher in ascorbic acid than the peripheral storage leaves.

Questions have also been asked as to how much loss of certain vitamins occurs when plant tissues are allowed to stand exposed to the air during the extraction of plant juices and the preparation of purées. Sliced bananas left exposed to the air were found by Harris and Poland (1939) to suffer a destruction of more than 10 per cent of their ascorbic acid in 20 minutes' standing, and in five hours they lost about 40 per cent of this vitamin. Curran, Tressler, and King (1937) found that apples cut into quarters lost about 20 per cent of their vitamin C in one hour of standing at room temperature, and 30 to 36 per cent in three hours.

Kohman, Eddy, and Gurin (1931) allowed whole and shredded carrots to stand exposed to the air and found that in the first hour of standing the shredded carrots lost more vitamin C than the whole ones, and in three hours of standing the effect was somewhat greater. Szent-Gyorgi (1931) found that the relatively large amount of "reduced hexuronic acid" present in intact cabbage leaves disappeared within the first five minutes after mincing. Also, when chard was chopped with a knife, it was found by Fenton, Tressler, Camp, and King (1937) to lose more ascorbic acid than when it was cut with the scissors, presumably owing to a greater bruising of the tissues.

The pressing of peaches to extract the juice has been shown by DeFelice (1942) to result in a loss of 50 per cent of the original vitamin A activity. Expressed vegetable juices have been found by Puffer, Hinman, Charley, and Halliday (1942) to contain from eight to 69 per cent of the reduced ascorbic acid, 22 to 36 per cent of the carotene, 15 to 90 per cent of the

calcium, and 24 to 62 per cent of the phosphorus of the whole vegetable. Claim has been made in the past that fruits and vegetables puréed while hot lost more ascorbic acid than when puréed cold. A recent study by Hauck (1943), however, failed to confirm this claim, finding instead that cold expression of tomato juice (with or without previous heating of the pulp) resulted in no better preservation of ascorbic acid in home-canned tomato juice than did expression of the juice from the hot pulp.

An interesting study by Koldt and Stieb (1937), has shown that the presence of cooking salt or white sugar retards the autoxidation of ascorbic acid in diluted lemon juice, either before or after heating for short periods of time. Todhunter and Robbins (1941) found that the addition of sugar to red raspberries in preparation for freezing appeared to have some protective action on the ascorbic acid but the authors did not consider their data conclusive.

Some investigations have been made concerning the extent to which soaking in cold water (preliminary to cooking) causes loss of nutrients by solution. Pfund and Nutting (1942) have found that the soaking of whole, pared potatoes for six hours in cold water did not cause significant increase in iron losses. A similar study concerning ascorbic acid in potatoes by Richardson and Mayfield (1943) has shown that long soaking of pared, quartered potatoes in "fresh" water before cooking caused some additional losses of ascorbic acid, but similar soaking in 2.5 per cent sodium chloride solution actually resulted in smaller losses of ascorbic acid than when potatoes were cooked immediately after preparation. Kelly and Porter (1941) found that when beans were boiled in the water in which they had been soaked for 16 hours, they showed no higher thiamin content than when they were boiled in fresh, distilled water after a similar period of soaking.

COOKING

In probably no other kitchen procedure is there as much variability in the methods used by different workers as there is in vegetable cookery. Standards with respect to what constitutes "doneness" in vegetables vary greatly, as do the techniques by which this end is achieved. As a result the nutritive value of cooked vegetables may be expected to differ widely. Studies reported in the literature show this to be the case.

Losses of calcium and phosphorus have been found in recent years to be much less than formerly believed possible. In studying about 15 different kinds of vegetables, Noble and Halliday (1937) found that with short periods of cooking—for example, cabbage boiled for six to eight minutes instead of 30 minutes as by Peterson and Hoppert (1925)—most vegetables retained at least 85 per cent of the original calcium and phosphorus when distilled water was used, and a larger amount of calcium than this when Chicago tap water (containing 30 p.p.m. of calcium) was used. Another study by Donald, Willard, and Brodie (1940) has also found that vegetables "properly cooked in water" tend to lose very little calcium.

One investigation by Pfund and Nutting (1942) found no significant difference between the total iron content of the flesh of raw potatoes, of potatoes steamed after paring, and of those boiled whole but peeled after

cooking. The iron content of potatoes so prepared was, however, significantly greater than that of pared potatoes boiled whole, boiled in quarters, or soaked whole for six hours previous to boiling.

One study by Jordon (1935) has indicated a possibility that the addition of sodium chloride may favor calcium retention in some instances, but the results were by no means conclusive. The study already referred to by Pfund and Nutting (1942) has shown that the addition of sodium chloride to the water in which pared potatoes were boiled had no significant effect on the amount of iron lost.

Oser, Melnick, and Oser (1943) have subjected peas, potatoes, carrots, and broccoli to the "old-fashioned" cooking procedures, representative of methods in which no attempt is made to retain the essential vitamins and minerals, and the resulting vitamin losses averaged 31 per cent. In using "new-improved" methods such losses from these vegetables were reduced to an average of 10 per cent. The losses of minerals in either case were not large, showing an average of 12 and five per cent for the "old-fashioned" and "new-improved" methods, respectively.

Probably more studies are reported concerning the ascorbic acid values of fruits and vegetables than any other nutrient. This is to be expected, since this vitamin is soluble in water and is unstable to many of the conditions encountered in the home care of these products. It has been pointed out by Fenton (1940) that if vegetables are prepared in such a way as to be high in ascorbic acid, they will be high in many other desirable qualities. Hence, the ascorbic acid content of vegetables can be used as something of a criterion of general quality for these products.

During a 15-minute cooking period, almost two-fifths of the original ascorbic acid content of carrots was found by Fenton, Tressler, Camp, and King (1938) to be extracted into the cooking water, but only 11 per cent of it was actually destroyed. When steamed, the carrots themselves contained 86 per cent of the original value of this vitamin. One study by Mack, Tapley, and King (1939) concerning snap beans showed that very little ascorbic acid was actually destroyed during 25 minutes of boiling, but about one-third of the original amount was extracted into the cooking water. Brown and Fenton (1942) found that parsnips boiled to tenderness without peeling and similar samples cut in pieces and cooked to tenderness in a pressure saucepan both lost approximately 10 per cent of their original ascorbic acid. In both the boiling and the steaming methods which they used, the parsnips cooked whole and unpeeled retained more ascorbic acid than did those which had been sliced or cut in pieces.

Concerning the loss of ascorbic acid in potato cookery, Esselen, Lyons, and Fellers (1942) found that all methods of cooking which they had studied caused some destruction of ascorbic acid of potatoes, the amount varying with the cooking method from 33 to 80 per cent. Boiling potatoes whole and unpeeled in salted water, baking, and French-frying seemed, from their results, to be the best methods for retention of ascorbic acid. However, a similar study by Richardson and Mayfield (1943) found that boiling potatoes whole in their jackets for 35 minutes caused no loss of ascorbic acid; and a similar cooking period, using pared, quartered potatoes, caused a loss of 18.7 per cent of this vitamin.

Wellington and Tressler (1938) have studied the losses of ascorbic acid in cooking cabbage. They boiled to tenderness cabbage which was prepared by shredding, by cutting in strips, and by quartering, and found that in all cases the amount of ascorbic acid actually destroyed was less than one-sixth of the original amount. When finely shredded cabbage was used, however, about two-thirds of the ascorbic acid was extracted into the cooking water, whereas with larger pieces of cabbage the amount extracted was less. In connection with summer squash, Woodruff and Scoular (1942) found that increase in loss of ascorbic acid content as cooking time increased was less when samples of this vegetable were garden fresh.

Floyd and Fraps (1940), in studying turnip greens, found that under conditions similar to those of "practical cooking" this vegetable lost 15.5 to 26.7 per cent of its ascorbic acid on "rapid cooking" and 23.8 to 36.5 per cent on "slow cooking."

Few studies have been reported concerning the effect of cooking on the thiamin in vegetables, but one investigation by Aughey and Daniel (1940) has shown that as much as 22 per cent of this vitamin may be destroyed during the boiling of some vegetables and, in addition, amounts up to 15 per cent may be lost in the cooking water. The addition of small amounts of sodium bicarbonate to the cooking water increased markedly the destruction of thiamin in green peas and snap beans but had no significant effect on the thiamin of boiled navy beans.

Little information is available concerning the effect of cooking operations on the nutritive value of fruits. Since rhubarb is used as a fruit, one study by Brown, Schuele, and Fenton (1941) may be mentioned here. When baked for 30, 35, and 40 minutes, it retained 88, 80, and 73 per cent, respectively, of its original ascorbic acid whether peeled or unpeeled, and the addition of sugar did not affect the ascorbic acid retention. Cooking rhubarb as a sauce, however, caused a loss of 30 to 40 per cent of this vitamin, according to Clague, Fellers, and Stepat (1936).

Curran, Tressler, and King (1937), in using Northern Spy apples, found that the unstrained sauce made from peeled apples showed a slightly greater retention (75 per cent) of the original ascorbic acid than the strained sauce made from unpeeled fruit (68 per cent). In the case of apple sauce prepared from peeled apples, the greatest loss of ascorbic acid took place during the first four minutes of cooking. Approximately 80 per cent of the original ascorbic acid was lost during the baking of apples and during the baking of apple pie.

Some attention has been given to the importance of the kind of container used in cooking vegetables, so far as nutrient losses are concerned. Studies have included such containers as aluminum, enamel, Pyrex, and stainless steel and have been reported by Floyd and Fraps (1940) and by Brown, Schuele, and Fenton (1941). Under some conditions it seems, from these reports, that the type of container may make some difference in nutrient retention but in no case did such difference seem large.

The studies mentioned so far have all been concerned with changes in percentage composition of various food constituents brought about by cooking. Equally important is any influence which cooking may have upon the availability of these constituents for nutritional purposes. Reporting

favorable effects in this connection are some studies concerning soybeans. Kelly and Porter (1941) found that the boiling and baking of dried soybeans increased the amount of thiamin available, and Baldwin and Movitt (1942) found that steam-autoclaving increased the nutritional value of the protein of soybeans 62 per cent above that of the raw soybean protein.

HOLDING AND REHEATING COOKED FOOD

Fewer studies have been reported concerning the effect of holding leftover food and reheating it than probably any other common kitchen practice, but the results of some of these are especially interesting. Esselen, Lyons, and Fellers (1942) found that after two days of storage in an electrical refrigerator, mashed potatoes had lost 40 per cent of their ascorbic acid when kept in covered dishes and 50 per cent when kept in uncovered ones. In view of the large losses reported in this one investigation, it is highly desirable that more data in this connection be accumulated. Pfund and Nutting (1942) found that a short period (15 minutes) of holding cooked, pared potatoes either in a dry pan or in the cooking water did not cause appreciable increase in iron losses. According to Curran, Tressler, and King (1937) apples in apple pie, which had lost 80 per cent of their ascorbic acid during baking, lost an additional eight per cent during a subsequent 48-hour period of standing in the pie.

SUMMARY

The nutritive value of fruits and vegetables can be altered materially by home procedures, and the extent of such effect differs greatly according to the care they receive. However, results of studies have been by no means consistent and, in some connections, data available are very incomplete.

Information concerning the nutritive value of fresh produce should be of special interest to growers to enable them to market samples of highest value according to variety, size, and stage of maturity. Some of this information is also helpful in the selection of fruits and vegetables from the market. In several instances, small, mature samples have been found to be higher in certain nutrients than large, mature ones of the same variety. In most studies reported concerning the degree of maturity, samples are found to increase in at least ascorbic acid content to a given degree of ripeness (often to the stage at which the sample is best for eating) and then to decrease as they become overripe. Leaf lettuce has been found to be higher in vitamin A than head lettuce and green lettuce leaves higher in vitamin A and in riboflavin than pale leaves.

The effect of home storage on the content of certain vitamins varies greatly according to the time and temperature of storage and also according to the sample itself. In practically all cases, storing samples at refrigerator temperatures has been found to cause significantly less loss of vitamin A and of ascorbic acid than storing them at room temperatures, and this difference has been especially pronounced when long periods of storage have been involved. The use of a humid atmosphere (as in a refrigerator hydrator) has been found to retard the destruction of vitamin A in lettuce.

Some operations preliminary to cooking have been found to be very wasteful of nutrients, whereas others have not been found to cause as

great losses as have been assumed in the past. Discarding the skins of some fruits and vegetables has been found to cause serious loss of nutrients, whereas in other cases the loss has not been great. The leaves of various greens have been found to be so much higher in iron than the stems, that it has seemed possible to discard the stems without incurring large iron losses. Also, the upper part of broccoli and rhubarb stalks have been found to be considerably more valuable as sources of ascorbic acid than the lower part of these stalks.

Allowing the cut surfaces of various kinds of fruits and vegetables to stand exposed to the air has been found to result in a reduction of ascorbic acid, and in some instances, at least, the extent of such reduction has depended upon the amount of bruising the plant tissues received. Wide variation has been found in the mineral and vitamin losses involved in the preparation of fruit and vegetable juices and purées. Potatoes left in large pieces have been found to lose little iron or ascorbic acid when soaked for considerable periods in cold water before cooking.

Losses in nutritive value which occur in cooking fruits and vegetables vary widely according to the degree of "doneness" acquired and the method of cooking. Shortened periods for vegetable cookery, as used in recent years, have resulted in decreased losses of at least certain nutrients. Since ascorbic acid is both water-soluble and unstable to the conditions encountered in cooking, vegetables tend to suffer losses of this nutrient during cooking; and conversely, cooked samples which are high in this nutrient may be expected to be high in others. Partly for this reason, no doubt, many studies are reported concerning the effect of different methods of cooking on the ascorbic acid content of vegetables. Losses have been found to differ according to the time of cooking, the amount of water used, and the size of pieces into which the vegetables were cut. Losses in calcium, phosphorus, and iron have been found in some cases to be much less than formerly believed possible. Little information is available concerning the effect of holding leftover food or of reheating it, but the few studies that have been reported indicate that vitamin losses resulting from these practices may be very considerable.

On the whole, generalizations that may be made concerning the effect of home practices on the nutritive value of fruits and vegetables are more limited than might be expected. As there come to be accumulated data from a sufficient number of laboratories indicating somewhat consistent results as to the effect of commonly used home procedures, it is to be hoped that recommendations will be given publicity so that a large number of consumers as well as research workers may benefit from them. Considered on a national scale, the extent of conservation of nutritive value so gained could be great.

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PALATABILITY STUDIES[1] OF COMMERCIALLY DEHYDRATED VEGETABLES

I. EFFECT OF SEVERAL METHODS OF STORAGE ON PALATABILITY OF BEETS, CABBAGE, AND RUTABAGAS

II. EFFECT OF SEVERAL COMMON REFRESHING AND COOKING METHODS ON PALATABILITY AND WATER ABSORPTION OF BEETS, CABBAGE, POTATOES, RUTABAGAS, AND YELLOW TURNIPS

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During the first World War vegetables were dehydrated for the use of our allies and our armed forces. Their palatability was considered satisfactory immediately after they were dried, but no palatability studies after storage were reported. Once again quantities of dehydrated products are being sent to our armed forces as well as to our allies for civilian use under the lease-lend program. Since the object of dehydrating foods is to preserve them for future use, it is vital to know the effect of various storage conditions on their palatability.

Quantitative tests have not been reported in the literature concerning the effect on their palatability and nutritive value of refreshing[2] dehydrated vegetables. Statements vary from recommendation of starting cooking in cold to boiling water and from no soaking to soaking overnight. The work reported in this paper was part of a study of the nutritive values of dehydrated vegetables carried out co-operatively with the U. S. Bureau of Home Economics, New York State Agricultural Experiment Station, the University of California, and the University of Texas.

This report is divided into two parts: (1) the effect of several methods of storage on the palatability of commercially dehydrated beets, cabbage, and rutabagas and (2) the effect of several common refreshing and cooking methods on the palatability and water absorption of commercially dehydrated beets, cabbage, potatoes, rutabagas, and yellow turnips.[3] The history of the vegetables used in this study as well as the losses of thiamin, carotene, and ascorbic acid during dehydration and storage are reported by Tressler, Moyer, and Wheeler (1943). The losses of these vitamins during cooking of vegetables from the same lots are reported by Fenton, Barnes, Moyer, Wheeler, and Tressler (1943). The moisture content of the dehydrated vegetables was beets, 5.5 per cent; cabbage, 5.5 per cent; potatoes, 7.0 per cent; rutabagas and yellow turnips, 8.4 per cent.

[1] The work reported in this paper was supported in part by the U. S. Bureau of Home Economics.

[2] Refreshing is the term generally applied to the preliminary soaking of dehydrated products.

[3] The vegetables were secured from the Dry Pack Corporation, Lyons, New York.

The conclusions reached in these studies are not necessarily applicable to vegetables of other varieties and maturities dehydrated by other methods and to other moisture levels or to those stored for different times or under different conditions.

I. EFFECT OF SEVERAL METHODS OF STORAGE ON PALATABILITY OF COMMERCIALLY DEHYDRATED BEETS, CABBAGE, AND RUTABAGAS

Palatability studies were conducted on the vegetables within a few days after they were dehydrated and again after five months' storage in three types of packages at four different temperatures. Each vegetable was packaged (1) in tightly closed glass containers, (2) under carbon dioxide in tightly closed glass jars, or (3) in either moistureproof cellophane or pliofilm bags. The storage temperatures were —40, .5, 14, and 24°C.(—40, 32.9, 57.2, and 75.2°F.). It has been found that frozen vegetables stored at —40°C. undergo very little change over a period of time, according to Jenkins, Tressler, and Fitzgerald (1938). Therefore, some of the dehydrated vegetables were stored at this temperature in order that they might serve as a control.

The color of the uncooked and cooked, dehydrated vegetables was determined by the use of Maerz and Paul color charts. All samples were judged for appearance, odor, flavor, and texture and in addition the cooked samples were scored for acceptability. The judges were asked to score the cooked, dehydrated vegetables on their own merits rather than on the basis of their resemblance to cooked, fresh vegetables of the same kind. All of the judges expressed themselves as preferring fresh vegetables which were cooked only to the firm, tender stage rather than to the "soft" stage.

Ten-gram samples were added to 150 ml. of boiling water. In previous tests where water at 22 and 98°C.(71.6 and 208.4°F.) was used the judges were not able to distinguish between the cooked products. The vegetables were cooked with no preliminary soaking. As soon as the water returned to the boiling point the flame was adjusted with a manometer so that the vegetables were simmered rather than boiled. They were cooked in one-quart enamel pans with glass covers. The pans had a diameter of five inches and depth of three and three-fourths inches. The cooking times were 12 minutes for cabbage, 25 minutes for beets, and 30 minutes for rutabagas.

EFFECT OF STORAGE CONDITIONS ON PALATABILITY OF UNCOOKED VEGETABLES

The palatability scores on the freshly dehydrated vegetables before and after cooking (Table 1) indicate that the effect of storage conditions (Table 2) was much more noticeable in the uncooked vegetables than in the cooked ones. The cooking process, in many cases, seemed to obliterate the changes noted in the uncooked product.

Beets: There seemed to be less effect on beets by changes in storage conditions than on cabbage or rutabagas. All samples had a similar appearance and a good, natural, dark-red color. The odor was also natural and similar throughout except in the case of the package stored at 24°C., which had an "off" odor and flavor. The flavor of the other samples was

TABLE 1

Palatability of Freshly Dehydrated Vegetables Before and After Cooking

Vegetable	Before cooking					After cooking [2]				
	Color		Odor	Flavor	Texture	Color		Odor	Flavor	Texture [3]
	Plate No.[1]	Description				Plate No.[1]	Description			
Beets	48J8	Natural dark red	Natural	Natural	Hard	7H66	Natural red	Natural	Natural	Natural
Cabbage	Lightest 17D1 Greenest 17G4	Slightly brown with a few green pieces	Natural	Natural	Crisp and brittle	17B1, 17C1, 17C2	Creamy	Natural	Natural	Natural
Rutabagas	10G6	Natural yellow	Natural	Natural	Hard	11I6, 11J6	Natural yellow	Natural	Natural	Natural

[1] Maerz and Paul color chart. [2] Ten grams of each dehydrated vegetable were added to 120 ml. of water and were cooked without refreshing for the following periods: beets, 25 min.; cabbage, 12 min.; rutabagas, 30 min. All were acceptable. [3] All the cooked vegetables had more "body" or chewiness than did the corresponding fresh ones. None were overcooked to the water-soaked stage.

characteristic and good, though slightly weaker, in the samples stored at 14 and 24°C. than in those stored at lower temperatures. The consistency was hard and good throughout, though slightly less so in the sample stored in the package at 24°C. Beets packaged in a sealed glass container had a much better odor and flavor when the jar was packed full than when it was about half-full.

Cabbage: The cabbage, which was of the Danish Ballhead variety, stored at —40°C. retained its original slightly brown color, the odor was natural, the flavor characteristically sweet, and the texture crisp. No differences owing to methods of packaging at this temperature were noticeable.

Cabbage stored at .5°C. had a good color with as much green in it as when stored at —40°C., but the lighter colors contained slightly more tan. Odor and flavor were similar to the —40°C. product, but the texture was not so crisp. This was particularly true of the moisture-vapor-proof-packaged sample which was more limp than were samples stored in air or carbon dioxide.

Cabbage stored at 14°C. in a moisture-vapor-proof package had a poor brownish color, hay-like odor, "off" flavor, and a limp, leathery texture.

Cabbage stored at 24°C. had a brownish color, an "off" odor and flavor, and a limp texture.

Rutabagas: Rutabagas stored at —40°C. in any type of package retained their original color, characteristic odor, natural, sweet flavor, and hard texture.

Those stored at .5°C. had an excellent bright color when stored in carbon dioxide, a somewhat less bright color, though still good, when stored in air and in moisture-vapor-proof bags. The odor was natural and flavor natural and sweet in all cases. Texture was good, though not quite so crisp as the —40°C. samples.

Those stored at 14°C. had a good appearance although the color was pale compared with that of the samples stored at lower temperatures. The sample stored in carbon dioxide had the best color. The odor was weak in the sample stored in carbon dioxide and "off" ("violet") in the samples stored in air and cellophane. The flavor was natural in the carbon dioxide samples but weak in the other two. The texture was crisp in the carbon dioxide samples but limp in the other two.

Rutabagas stored at 24°C. had a fair to poor appearance. The samples stored in carbon dioxide were the best; the other two were similar to that in the bag stored at 14°C. Odor was "off" ("violet"), flavor lacking, and texture limp in all cases.

EFFECT OF STORAGE CONDITIONS ON PALATABILITY OF COOKED VEGETABLES

Beets: No differences in palatability in any of the beet samples were discernible after cooking. In all cases the color was natural and excellent, and the odor, flavor, and texture were characteristic.

Cabbage: No differences were discernible in the cooked cabbage which had been stored at —40 and .5°C. in the various containers. All samples retained the original slightly brown color, natural flavor, odor, and texture.

TABLE 2

Effect of Different Storage Methods for a Five-Months Period on Palatability of Dehydrated Vegetables Before and After Cooking

Vegetable	Storage		Before cooking					After cooking[1]					
			Color					Color					
	Temperature	Package	Plate No.[1]	Description	Odor	Flavor	Texture	Plate No.[1]	Description	Odor	Flavor	Texture	Acceptability
	°C.												
Beets..........	—40	Air	56C11	No	No	Natural	Hard	7H6	No				
		CO_2	56C11			Natural	Hard	7H6		No	No	No	
		Pkg.	56C11			Natural	Hard	7H6					All
				observable	observable				observable	observ-	observ-	observ-	
	.5	Air	56C11			Natural	Hard	7H6					were
		CO_2	56C11			Natural	Hard	7H6		able	able	able	
		Pkg.	56C11	change	change	Natural	Hard	7H6	change				very
										change	change	change	
	14	Air	56C11			Weak	Hard	7H6					accept-
		CO_2	56C11	during	during	Weak	Hard	7H6	during	during	during	during	
		Pkg.	56C11			Weak	Hard	7H6					able
										storage	storage	storage	
	24	Air	56C11	storage	storage	Weak	Hard	7H6	storage				
		CO_2	56C11			Weak	Hard	7H6					
		Pkg.	56C11		Off	Off	Less hard	7H6			Poor		
Cabbage:.....	—40	Air	17D1 & 17G4	Slightly	Natural	Natural	Crisp	10B1	Creamy tan	No	Natural	No	Good
		CO_2	17D1 & 17G4	tan with	Natural	Natural	Crisp	10B1	Creamy tan		Natural		Good
		Pkg.	17D1 & 17G4	some green	Natural	Natural	Crisp	10B1	Creamy tan	observ-	Natural	observ-	Good
	.5	Air	17D1 & 17G4	Similar to	Natural	Natural	Less crisp	10B1	Creamy tan	able	Natural	able	Good
		CO_2	17D1 & 17G4	above but	Natural	Natural	Limp	10B1	Creamy tan		Natural		Good
		Pkg.	18D1	more tan &	Natural	Natural	Limp	10B1	Creamy tan	change	Natural	change	Good
				less green									

TABLE 2 (*Concluded*)

Cabbage......	14	Air		Tan						No observable change			
		CO_2		Tan									
		Pkg.	9D3	Tan	Hay-like	"Weedy" and off	Limp	11C4	Darker tan		Weak		Poor
	24	Air	9D3 trace 17D1	Darker tan	Hay-like	"Weedy" and off	Limp	11C4	Darker tan		Weak		Poor
		CO_2		Darker tan	Hay-like	"Weedy" and off	Limp	11C4	Darker tan		Weak		Poor
		Pkg.		Darker tan	Hay-like	"Weedy" and off	Limp	11C4	Darker tan		Weak		Poor
Rutabagas..	—40	Air	10G6	Natural yellow	Natural	Natural	Hard	10G5	Natural yellow	No observable change	Natural	No observable change	All acceptable although some observable differences in color and flavor
		CO_2	10G6	Natural yellow	Natural	Natural	Hard	10G5	Natural yellow		Natural		
		Pkg.	10G6	Natural yellow	Natural	Natural	Hard	10G4	Natural yellow		Natural		
	.5	Air	10G5	Lighter yellow	Natural	Natural	Hard	10G3	Lighter yellow		Weak		
		CO_2	10G6	Lighter yellow	Natural	Natural	Hard	10G4	Natural yellow		Natural		
		Pkg.	10G5	Lighter yellow	Natural	Natural	Hard	10G3	Lighter yellow		Weak		
	14	Air	11F7	Grayish yellow	Off	Weak	Limp	10G4	Lighter yellow		Weak		
		CO_2	11I8	Lighter yellow	Weak	Natural	Hard	10G5	Natural yellow		Natural		
		Pkg.	11F4	Grayish yellow	Off	Weak	Limp	10G3	Grayish yellow		Weak		
	24	Air	11F4	Grayish yellow	Off	Weak	Limp	10F3	Grayish yellow		Weak		
		CO_2	11F7	Lighter yellow	Off	Weak	Limp	10F3	Grayish yellow		Weak		
		Pkg.	11F4	Grayish yellow	Off	Weak	Limp	10F3	Grayish yellow		Weak		

[1] Ten grams of each dehydrated vegetable were added to 120 ml. of water and were cooked without refreshing for following periods: beets, 25 min.; cabbage, 12 min.; rutabagas, 30 min. All were acceptable.

Palatability decreased markedly between .5 and 14°C. storage. In fact, the cabbage stored at 14°C. in a moisture-vapor-proof bag was rated not acceptable. The color was brown and the flavor weak; the odor and texture were natural.

All the samples stored at 24°C. were similar and were rated not acceptable. The color was brown, the odor and texture were natural, but the flavor was weak.

Rutabagas: The most noticeable difference in the cooked samples of rutabagas was the fading of color as the storage temperature increased. The effect was less marked in the samples stored in carbon dioxide. Odor and texture were natural. The flavor was weak in all 12 samples.

II. EFFECT OF SEVERAL COMMON REFRESHING AND COOKING METHODS ON WATER ABSORPTION AND PALATABILITY OF COMMERCIALLY DEHYDRATED BEETS, CABBAGE, POTATOES, RUTABAGAS, AND YELLOW TURNIPS

For the reasons stated in the introduction, refreshing tests in which temperature of the water and length of the soaking time were increased, were run on commercially dehydrated beets, cabbage, potatoes, rutabagas, and yellow turnips. These studies included (1) the amount of water reabsorbed during (a) refreshing and (b) cooking, and (2) palatability tests on the cooked vegetables. Because vitamin C is the most easily destroyed of all the known vitamins and since no other vitamin is more easily dissolved, a study was made of its losses during the refreshing procedures. Vitamin C determinations were made on cabbage only, because it was the one vegetable in the group which contained sufficient vitamin C for the study. As previously stated, the cabbage had not been blanched previous to dehydration; consequently the results would not necessarily apply to cabbage which was blanched previous to dehydration.

REABSORPTION

Tap water was used in all of the studies because earlier work in this laboratory showed no observable differences in reabsorption or in palatability scores when tap and distilled water were used.

Ten-gram samples of the dehydrated vegetables were cooked directly or were soaked in a measured quantity of tap water. The amount of water used for each vegetable (Table 3) was enough to permit maximum rehydration at the end of the cooking period but not enough to allow an excess of water. The amount of water remaining at the end of the cooking period after seven hours' soaking varied from one gram with rutabagas to 80 grams with cabbage. The time of refreshing varied from none to seven hours. In preliminary tests, soaking for periods longer than seven hours had very little further effect on the amount of water reabsorbed, hence the results of such tests were not included in this study. For each period duplicate samples were started soaking in water at 20, 80 and 98°C. (68, 176, and 208.4°F.). These temperatures were not maintained so the water gradually came to room temperature. Samples were drained and weighed after soaking, and the remaining water was measured. Then the samples were cooked, again drained and weighed, and the remaining water measured. The cooking time for each vegetable was determined by preliminary

TABLE 3

Effect of Increasing Time and Temperature of Refreshing on Reabsorption of Water During Refreshing and Cooking[1] of Dehydrated Vegetables[1] and on Their Palatability

Vegetable	Refreshing time	Temperature of water	Water absorbed after		Palatability					
			Refreshing	Cooking	Color		Odor	Flavor	Texture	Acceptability[3]
					Plate No.[2]	Description				
	hr.	°C.	pct.	pct						
Beets	None	20	....	450	47L3	Natural	Natural	Natural	Natural	Yes
		80	....	430	47L3	Natural	Natural	Natural	Natural	Yes
		98	......	460	47L3	Natural	Natural	Natural	Natural	Yes
	½	20	350	490	47L3	Natural	Natural	Natural	Natural	Yes
		80	360	480	47L3	Natural	Natural	Natural	Natural	Yes
		98	420	510	47L3	Natural	Natural	Natural	Natural	Yes
	1	20	430	530	47L3	Natural	Natural	Natural	Natural	Yes
		80	450	520	47L3	Natural	Natural	Natural	Natural	Yes
		98	430	540	47L3	Natural	Natural	Natural	Natural	Yes
	2½	20	520	560	55L1	Pale[4]	Natural	Weak[4]	Water-soaked[4]	No[4]
		80	470	520	55L1	Pale[4]	Natural	Weak[4]	Water-soaked[4]	No[4]
		98	530	550	55L1	Pale[4]	Natural	Weak[4]	Water-soaked[4]	No[4]
	7	20	560	590	55L1	Pale[4]	Natural	Very weak	Water-soaked[4]	No[4]
		80	530	540	55L1	Pale[4]	Natural	Very weak	Water-soaked[4]	No[4]
		98	550	580	55L1	Pale[4]	Natural	Very weak	Water-soaked[4]	No[4]
Cabbage	None	20	......	760	19C1	Natural	Natural	Natural	Natural	Yes
		80	......	720	19C1	Natural	Natural	Natural	Natural	Yes
		98	......	730	19C1	Natural	Natural	Natural	Natural	Yes
	½	20	680	790	19C1	Natural	Natural	Natural	Natural	Yes
		80	700	790	19C1	Natural	Natural	Natural	Natural	Yes
		98	720	800	19C1	Natural	Natural	Natural	Natural	Yes
	1	20	740	820	19C1	Natural	Natural	Natural	Natural	Yes
		80	690	800	19C1	Natural	Natural	Natural	Natural	Yes
		98	730	820	19C1	Natural	Natural	Natural	Natural	Yes

TABLE 3 (Continued)

	2½	20	750	800	19C1	Pale [4]	Weak [4]	Weak [4]	Water-soaked [4]	No [4]
		80	750	830	19C1	Pale [4]	Weak [4]	Weak [4]	Water-soaked [4]	No [4]
		98	750	820	19C1	Pale [4]	Weak [4]	Weak [4]	Water-soaked [4]	No [4]
	7	20	780	800	19C1	Pale [4]	Weak [4]	Very weak	Very water-soaked [4]	No [4]
		80	740	810	19C1	Pale [4]	Weak [4]	Very weak	Very water-soaked [4]	No [4]
		98	770	800	19C1	Pale [4]	Weak [4]	Very weak	Very water-soaked [4]	No [4]
Potatoes...............	None	20	......	380	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		80	......	430	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		98	......	420	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
	½	20	230	430	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		80	290	420	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		98	320	440	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
	1	20	290	430	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		80	390	480	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
		98	370	460	10A1 to 10B1	Natural	Natural	Natural	Natural	Yes
	2½	20	330	460	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	390	460	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	380	460	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
	7	20	380	490	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	390	480	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	410	480	10A1 to 10B1	Natural	Natural	Weak [4]	Water-soaked [4]	No [4]
Rutabagas...........	None	20	......	560	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		80	......	550	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		98	......	550	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
	½	20	420	580	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		80	530	660	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		98	540	650	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
	1	20	530	640	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		80	620	680	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes
		98	600	680	11G7 to 11F1	Natural	Natural	Natural	Natural	Yes

TABLE 3 (*Concluded*)

Effect of Increasing Time and Temperature of Refreshing on Reabsorption of Water During Refreshing and Cooking[1] of Dehydrated Vegetables[1] and on Their Palatability

Vegetable	Refreshing time	Temperature of water	Water absorbed after		Palatability					
					Color					
			Refreshing	Cooking	Plate No.[2]	Description	Odor	Flavor	Texture	Acceptability[3]
	hr.	*°C.*	*pct.*	*pct.*						
Rutabagas..........	2½	20	770	770	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	780	790	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	770	780	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
	7	20	990	910	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	1040	970	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	960	910	11H5 to 11F3	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
Yellow turnips...	None	20	......	520	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		80	......	500	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		98	......	530	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
	½	20	410	590	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		80	430	640	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		98	490	610	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
	1	20	490	650	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		80	500	650	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
		98	480	640	11I4 to 11G2	Natural	Natural	Natural	Natural	Yes
	2½	20	560	690	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	680	680	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	560	690	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
	7	20	750	820	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		80	870	900	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]
		98	760	830	11F2 to 11C1	Pale	Natural	Weak [4]	Water-soaked [4]	No [4]

[1] Ten grams of each dehydrated vegetable (stored three to four months) were added to 120 ml. of water. The cooking times were for beets, 25 min.; cabbage, 12 min.; potatoes, 20 min.; rutabagas and turnips, 30 min. [2] Maerz and Paul color charts. [3] No noticeable differences owing to the temperature of the water. Palatability, particularly flavor, was best with no soaking and for one-half hour of soaking, grading to poorest with seven hours' soaking. All of the long-soaked vegetables were rated not acceptable because of pale color, weak flavor, and watery texture. [4] At this point the change was very marked although it had been occurring

tests. The cooking process was kept uniform throughout the study. All samples were cooked in one-quart enamel pans with glass covers. The amount of evaporation during cooking was kept constant by a manometer between the burner and the gas outlet—Fenton, Tressler, Camp, and King (1937). All refreshing tests were run in duplicate.

Palatability: Tests for palatability, made on the final cooked products, included comparisons of color, odor, flavor, consistency, and acceptability (Table 3). All scores were made by the same judges. Color was described by the use of the Maerz and Paul color charts.

Vitamin Determinations: The vitamin C determinations were made by a modified Bessey and King (1933) procedure, according to Mack and Tressler (1937). The vitamin C determinations were made on the uncooked, dehydrated cabbage and on the cooked, dehydrated cabbage which had been put in water at 20 and 98°C.(68 and 208.4°F.) and cooked with no preliminary soaking. They were also made on cabbage which had been cooked after preliminary soaking of 30 and 60 minutes at each of the above temperatures.

RESULTS

Effects of Temperature of Water: Allowing the vegetables to soak or to cook in water at 20, 80, or 98°C. made very little difference in the amount of water absorbed and no observable difference in the color, odor, flavor, or consistency of the cooked vegetables (Table 3). In fact, the judges in no case were able to distinguish differences in the palatability of any vegetable placed in water at 20, 80, or 98°C. and allowed to stand at room temperature. There was a difference, however, in the retention of vitamin C in cabbage. The unblanched, dehydrated cabbage put on to cook in boiling water retained 71 per cent of its vitamin C which was 43 per cent more than that retained by cabbage put on to cook in cold water (28 per cent). The increase in loss was due chiefly to destruction, which was 11 per cent in the former case and 60 per cent in the latter case. The per cent solution was about the same whether boiling or cold water was used, 18 and 12 per cent, respectively.

Effect of Refreshing Time: The effect of length of refreshing period on amount of water absorbed varied considerably with the kind of vegetable (Table 3). There was no appreciable difference in the amount of water absorbed in cabbage or potatoes whether unsoaked or soaked for as long as seven hours before cooking. It should be kept in mind that all of these vegetables were cut in small pieces. In other studies made in this laboratory, potatoes cut in wedges and in slices required from 20 to 60 minutes of soaking before cooking. Beets which had been soaked one-half hour before cooking absorbed more water than did those cooked unsoaked, but there was very little further increase with as long soaking as seven hours. The amount of water absorbed by rutabagas and yellow turnips increased gradually with increased soaking time.

In all cases palatability, particularly flavor, was judged highest for the unsoaked vegetables and those soaked for short periods, and lowest for those which were soaked the longest time (Table 3). There was a gradual decrease in the palatability with an increase in the refreshing time, par-

ticularly after 30 minutes' soaking. Beets, rutabagas, and turnips lost color very markedly with long soaking.. Soaking had no effect on the color of potatoes and cabbage. With all the vegetables studied, the longer the soaking the weaker the flavor.

With every marked increase in water absorption there was a decrease in the desirability of the texture. With high water absorption the vegetables became water-soaked and heavy. This, with the lack of flavor and in many cases color, gave products of poorer palatability. Consequently, it may be desirable to prepare and dehydrate certain vegetables in such ways that they do not require soaking preparatory to cooking. In other tests made in this laboratory it has been found advisable from the texture standpoint, particularly, to cook leafy vegetables without soaking them.

The amount of vitamin C retained in the unblanched, dehydrated cabbage decreased progressively as the soaking time in cold water increased. Cooking after zero, 30, and 60 minutes of soaking, the decrease was 28, 19, and 7 per cent, respectively; the destruction was 60, 78, and 90 per cent, respectively. As stated previously, the enzymes in this cabbage had not been inactivated by blanching previous to dehydration.

In another study in this laboratory by Fenton, Barnes, Moyer, Wheeler, and Tressler (1943) it was found that varying the temperature of the water in which the vegetables were placed made no appreciable difference in the percentage of thiamin retained in the vegetables nor did cooking without refreshing or after refreshing for 30 minutes.

SUMMARY

The effect of storage conditions on the dehydrated cabbage, beets, and rutabagas used in this study was more noticeable in the uncooked than in the cooked vegetables.

Storage conditions had little effect on the palatability of cooked, dehydrated beets, but they did affect the palatability of cooked, unblanched, dehydrated cabbage markedly. All samples of dehydrated cabbage stored at —40 and .5°C.(—40 and 32.9°F.) were equally good, but samples stored at 14 and 24°C.(57.2 and 75.2°F.) for five months acquired a brown color and lost flavor. Storage conditions affected the palatability of cooked, dehydrated rutabagas in respect to color, mainly, and flavor, slightly. The bright, natural color faded as the storage temperatures increased. This effect was less marked in the samples stored in carbon dioxide.

Storage temperatures brought about more noticeable differences than did the method of packaging. At .5°C. the type of packaging was not so important as it was at the higher temperatures, and at —40°C. it was not a noticeable factor.

Differences in the samples stored at any one temperature were less in those stored in carbon dioxide. Of the three vegetables studied, the unblanched, dehydrated cabbage was most affected by methods of storage. Beets were the only one of the three vegetables studied that was stored at ordinary room temperatures for five months without loss of palatability.

The initial temperature of water, 20, 80, and 98°C., in which beets, cabbage, potatoes, rutabagas, and yellow turnips were soaked or cooked had very little effect on the amount of water reabsorbed or on the palata-

bility of the cooked vegetables. Unblanched, dehydrated cabbage started cooking in boiling water retained 71 per cent of its vitamin C; that started cooking in cold water retained only 43 per cent.

The effect of length of the refreshing period upon amount of reabsorption varied with the vegetable. In the case of cabbage and potatoes the increase was relatively small. Beets soaked for one-half hour before cooking absorbed more water than did those cooked with no soaking, but further increase in absorption was slight in beets soaked as long as seven hours. The amount of water absorbed by rutabagas and yellow turnips increased gradually with an increase in refreshing time. Unblanched, dehydrated cabbage put in cold water and cooked after zero, 30, and 60 minutes of soaking retained 28, 19, and 7 per cent of its vitamin C; the destruction was 60, 78, and 90 per cent, respectively. Long soaking resulted in water-soaked vegetables, loss of flavor, and often loss of color.

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ACTION OF HARDWOOD SMOKE ON BACTERIA IN CURED MEATS

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The first taste for smoked provisions probably arose from the ancient custom of suspending meats, fish, cheese curds, fowl, etc. near the smoke vent of a tent or the roof of a cave dwelling where foods were hung out of reach. Smoking of foods is an old practice with practically all the peoples of the earth and the custom extends far back into prehistoric times. It was noticed that when foods were penetrated by the vapors and smoke, their keeping qualities were greatly increased.

Rideal (1903) writes, "In northern climates the smoking of meats and fish was always practiced and oak or beechwood smoke was preferred, as these yield more of the preserving agents, acetic acid and creosote. The formation of an empyreuma or tarry liquor from wood was early known, and to hasten the process the flesh was dipped in it before smoking, but in this way an inferior product was obtained."

Glauber, in 1650, mentioned the preservative action of wood tar or the "oily part of the distillation of wood which is more fixed than the acid," and stated that if meat is washed, dried, and placed in strong vinegar, or boiled in a weaker vinegar, it will often keep for several months upon storage in a cool place. Fish and meat were "soused" or pickled in vinegar and spices in very early times.

At this point we wish to emphasize the fact that "liquid smoke" is never used in commercial establishments. There is a widespread belief that commercial manufacturers use "liquid smoke," as noted by Chenoweth (1930), but this is not the case. These solutions cannot be used in meat plants under Federal inspection. Furthermore, it has been known for many years that "liquid-smoke" solutions will not produce the preservative effect of hardwood smokes. As Ostertag and Young (1934) write, "since ancient times the preservative action of smoke has been used for retaining the durability of meat . . . liquid smoke will not produce the preservative effect of smoke . . . smoking causes shrinking of the muscle fibers and widening of the interstitial spaces."

There are many desirable effects to be gained by proper smoking of cured meats: (1) drying effect, (2) imparting desirable organoleptic properties, (3) bringing out color inside the cured meat, (4) imparting antioxidants to the fat, (5) impregnating the outside portions of the meat with constituents of smoke which serve as antiseptics and germicides, (6) if above 49°C.(120°F.), an adjuvant action of constituents of smoke and heat on microorganisms, (7) a tendering action from increased activity of autolytic enzymes of meat at temperatures above 15.6°C.(60°F.), (8) a "tenderizing" action from high humidity-high temperature smokehouse, (9) a diminution in nitrite content probably by reason of the aliphatic diazo reaction occurring at higher temperatures, (10) imparting a desir-

able "finish" or gloss on the skin-flesh sides of meat through the agency of aldehyde-phenol condensed resins from the smoke and the film of grease remaining on the piece.

SMOKEHOUSE AND SMOKE GENERATION

Smokehouses are usually placed adjacent to the curing and smoked-meat shipping departments. The walls are constructed of thick brick or tile, insulated with cork, and covered with a smooth surface of cement. The doors on each floor of the house are close fitting and fireproof. Smokehouses are several stories high, usually three floors and more. The ceiling is constructed so that condensates do not form, and a ventilator vents the smoke to the atmosphere through draft controls. The fire pit is situated at the bottom of the house about eight feet below the first smoking level. The pit contains a door for ventilation and for bringing sawdust and wood to the pit. Iron gratings serve as a floor for each level of the house.

There are several methods of firing a smokehouse. The old method utilized hardwood logs which were fired to heat the house; the fire was then smudged with sawdust. Newer methods utilize steam heating and development of the smudge by the use of gas flames directed against piles of sawdust. This reduces the amount of hardwood needed, and the scarcity of hardwood has caused many changes in the methods of generating smoke. Another method calls for oil burning units (without steam coils) but, of course, using hardwood sawdust. Charcoal briquettes are also used for heating and smudging sawdust. Temperature controls aid the smokehouse foreman to regulate the smoking temperatures with exactness so that all meats can be smoked according to "instructions." The house is kept clean by scraping and cleaning with caustic solutions and then washing with hot water under pressure.

CHEMICAL COMPOSITION OF WOOD SMOKE

Practically all of the early investigators in this field attributed the preserving action of smoke on meats to phenols, acetic acid, and aldehydes. The views held at the present time in the packing industry are that the smoke from hardwoods (hickory preferred) exerts a very definite preserving action, and the action is due to the aldehydes, phenols, and aliphatic acids. Coal, peat, pine, and most softwoods are useless because of the unpleasant taste imparted to the food, Ostertag and Young (1934). Pine smoke may impart turpentine to the food. In Europe, juniper, beech chips with juniper berries, sumach with chips of mahogany, oak, or beech are all used in the smokehouse processing.

The chemistry of wood smoke has not been studied extensively. Callow (1927) found that smoked ham and bacon gave a strong reaction for aldehyde. Hurd (1929) lists the compounds of destructive distillation of wood at atmospheric pressure as follows:

1. **Acids:** formic to caproic, especially acetic acid, some formic, furoic and angelic, lignoceric acids, and valerolactone.
2. **Alcohols:** methanol, allyl alcohol, isoamyl, and isobutyl alcohols.
3. **Ketones:** acetone, diacetyl.
4. **Aldehydes:** formic, acetaldehyde, dimethyl acetaldehyde, furfural.
5. **Phenols:** derived largely from the lignin.

6. Other constituents: ammonia, melene, m-xylene, toluene, methylpyridine, pyridine, CO_2, methane, carbon monoxide, esters, p-cresol, moisture. (Also guaiacols, vanillin, and aromatic substances.)

Recently Pettet and Lane (1940), in an excellent study of wood-smoke chemistry, found that the condensates recovered from the adsorbent by extraction with methylene dichloride contained formaldehyde, acetaldehyde, furfuraldehyde, 5-methyl furfuraldehyde, acetone, diacetyl, methyl and ethyl alcohols, phenol, formic, and acetic acids, resins, and wax. Some of the resinous material is formed by interaction of the phenols with formaldehyde. All smokes were deficient in phenols and in aliphatic acids as compared with the product of destructive distillation of wood. The reduction of phenol is accompanied by a lessening in formaldehyde and a marked increase in resinous compounds.

The glossy surface of properly smoked meats may result from these condensation products, i.e., the resins. The aldehydes, also, may produce a glossy appearance on the skin surface of meat when the meat has "oiled out" a little. The "wet, heavy smokes" produced by lower rates of combustion produce the best glosses or finish.

An ordinary hardwood smudge in large commercial smokehouses will show the following range of concentration of constituents in the smoke (based largely upon the calculations of Pettet and Lane (1940): formaldehyde, 25 to 40 p.p.m.; higher aldehydes, 140 to 180 p.p.m.; formic acid, 90 to 125 p.p.m.; acetic and higher acids, 460 to 500 p.p.m.; phenols 20 to 30 p.p.m.; ketones, 190 to 200 p.p.m.; resins, over 1,000 p.p.m. All smokes appear to contain less of the aldehydes, phenols, and aliphatic acids than the product of destructive distillation of wood. Heavy smoke carries more moisture, and probably causes less shrink of meats, but the factors of smokehouse temperatures, relative humidities, along with the variables of smoke (airflow, concentration), and time in smoke need considerable attention from the point of view of economy. That is to say, the maximum preserving action on meats gained from ideal smokehouse treatment needs to be followed carefully.

BACTERICIDAL ACTION OF SMOKE

Hess (1928) observed that formaldehyde vapors as found concentrated in smoke—three or more grams per 100 cubic feet—have a decided bactericidal action. Tanner (1932) states that while it is quite definitely known that formaldehyde in smoke is germicidal, it is also possible that other constituents of smoke may also be germicidal.

We know that the other constituents of smoke penetrate the meat and act in a desirable manner. Tucker (1942) worked out a chemical method for determining the distribution of phenols in meat and fat of smoked hams. While he does not indicate the smokehouse treatment of his samples, he does state that the hams were commercial hams smoked for 24 hours and analyzed seven days later. Each sample for chemical analysis weighed 50 grams; the results are expressed in terms of phenol (Table 1).

Tucker uses the phenol content as an indicator of the extent of smoke penetration.

Edelmann, Mohler, and Eichhorn (1939), who have long been experienced in meat hygiene, write as follows:

"The conserving effect of smoking in meat depends upon the extraction of water and the penetration of the meat with gases and fumes of the smoke, which are substances preventing putrefaction. Among these are the tar products and hydrocarbons insoluble in water; also acetic acid, creosote phenol, CO_2, NH_4OH, etc. Concerning the action of smoking on microörganisms, the investigations of Beu, Serafini, and Ungaro, showed that even pathogenic germs are destroyed in a short time if they are easily reached by the smoke. In the smoking of infected meat, it may be remembered, however, that a coagulated layer soon forms on the surface making the penetration of the smoke more difficult. Therefore, the germs contained on the inside of large pieces of meat may be destroyed only with difficulty. This is also influenced by the water content of the meat as the water prevents the penetration of the smoke."

Some interesting experiments on the effect of smoke on bacteria were performed by Hess (1928), who used an arbitrary scale of "cubic foot of air admitted to the smoke producer per hour" which gave a fair indication of smoke concentration. Hess used cultures of Staphylococci, *Proteus vulgaris,* and a sporeforming species of genus Bacillus. He abstracted his results as follows:

"An increase of the amount of air admitted per hour caused uniformly a greater reduction of bacteria, i.e., an increase of the bactericidal power of the smoke as well as a higher density of the smoke. Smoke of hardwood,

TABLE 1

Phenol Content of Smoked Ham

(After Tucker)

Description	Phenol content
	mg./100 gm.
Fat just below skin	0.45
Exposed fat not covered by skin	1.89
Lean surface tissue	1.36
Lean tissue ½ inch below lean surface	0.32
Lean tissue beneath fat layer	<0.03
Lean tissue in center of ham	<0.03

softwood, and mixtures of both, showed no difference in bactericidal power after six hours' action on the cultures. The rate of reduction of nonsporeformers was greatest between one-half to two hours' exposure to fairly weak smoke, with an initial weakening of the bacteria during the first half-hour, and killing of the more resistant forms during the third hour of exposure. The more concentrated the smoke, the greater were the initial and total rates of reduction. Spores were very resistant to smoke, the resistance increasing with increasing age of the culture; 71 per cent of the spores from a seven-months-old culture survived dense smoke seven hours, while nonsporing bacteria were killed in two hours by the same smoke."

The more concentrated the smoke, the higher its penetrating power. Smoke constituents which had been absorbed during the exposure continued to exert a bactericidal action after the smoking was finished. Formaldehyde vapors, containing three or more grams of HCHO per 100 cu. ft. (concentration in smoke), have a decided bactericidal action, and Hess considers it one of the chief bactericidal constituents of smoke.

In the presence of proteins, smoke was less effective on bacteria, but in dense smoke this effect was not as marked as in thin smoke. The bac-

tericidal action is greatest in acid medium. The resistance of bacteria against smoke is greatest in media of optimum salt concentration.

The Canadian workers, White, Gibbons, Woodcock, and Cook (1942), made bacteriological, chemical, and physical examinations on smoked and unsmoked Wiltshire bacon. They observed that smoking reduced the number of surface bacteria to approximately 10^{-4} times the number present before smoking and effectively retarded growth during storage. The fat of smoked bacon was more resistant to rancidity than unsmoked bacon. From the reduction in bacterial numbers, rate of bacterial growth, and rate of rancidity development, it would appear on the average that smoked bacon would keep about twice as long as unsmoked bacon under comparable conditions. In this connection, Lea (1933) has shown that smoking enables surface fat of bacon to resist oxidation for considerable periods of time.

STUDIES ON SMOKING OF CURED MEATS

Dr. W. M. Urbain, of Swift & Company Research Laboratories, followed several lots of cured, skinned bacon bellies from the wash, through the smokehouse, and up to the slicing operation. Samples of bacon were taken:

1. At the time of hanging in the smokehouse.
2. At the time the internal temperature of the bellies reached 100°F. (2½ hours).
3. At the end of 14 hours of smoke and then chilled.
4. After 18 hours of smoke.
5. Again after 18 hours in smokehouse and subsequently chilled.
6. After smoking for 18 hours and hanging for six hours in the smokehouse.

Samples of the bacon were taken as follows: Four inches were cut off the bottom of the belly and the next inch was taken as the sample. Two slices were cut off for color inspection. One inch of the belly was cut off from the center and two slices from each adjacent piece were also taken for color inspection Bacteriological examinations were made from the one-inch strips. Sodium nitrite analysis was also made from these one-inch strips. Color inspection was made by boiling the slices, noting the completeness of fixation of the pigment of the lean (listed in the tables as percentage), and noting the degree of color (listed by letter, G for good, F for fair, and P for poor). In Tables 2 to 7 are shown the place of sampling, the time of hanging in the smokehouse, and time smoked; the per cent of nitrite present at each period in the various places in the belly; the per cent and degree of color fixation; the total number of aerobic bacteria per gram on nutrient agar plates incubated for 72 hours at 20°C. (68°F.); the oxidizing bacteria and the hydrogen sulphide bacteria which discolor the cured pigments of bacon, Jensen (1942). The smokehouse air temperatures were 55.6 to 57.2°C. (132 to 135°F.).

Three additional tests, as listed above, were performed with practically the same results, i.e., low nitrite, good color, and few bacteria at the end of 18 hours in the smokehouse.

From these data it may be concluded that the sodium nitrite content does not increase during the smoking but steadily decreases. There is no reduction of the nitrate present to nitrite. The nitrite, according to W. Lee Lewis quoted by Winton and Winton (1937), disappears, and the destruction of nitrite when cured meat is heated comes about from an

TABLE 2

Some Effects of Smoking on Cold Bacon Samples Taken at Time of Entry Into Smokehouse, 7.2°C.(45°F.)

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
1. Middle	.0036	100 F	2,000,000	5,000	600,000
End	.0084	100 F	3,500,000	120,000	320,000
2. Middle	.0048	95 G	2,000,000	5,000	300,000
End	.0078	95 G	560,000	7,000	....
3. Middle	.0048	95 G	280,000	6,000	100,000
End	.0078	100 F	3,100,000	2,500	600,000
Average	.0062	....	1,900,000	28,000	400,000

TABLE 3

Samples Taken in Two and One-Half Hours When Internal Temperatures Reached 37.8°C.(100°F.)

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
4. Middle	.0030	90 G	720,000	120,000	400,000
End	.0078	100 F	2,300,000	1,000	1,000,000
5. Middle	.0030	95 G	1,500,000	0	900,000
End	.0036	100 F	500,000	8,000	300,000
6. Middle	.0030	98 G	480,000	1,000	140,000
End	.0034	100 F	3,000,000	0	450,000
Average	.0048	....	1,400,000	22,000	530,000

TABLE 4

Samples Taken After 14 Hours in Smokehouse and Subsequently Chilled[1]

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
7. Middle	.0009	100 G	820	0	500
End	.0009	100 G	620	50	150
8. Middle	.0015	100 G	25,000	1,000	17,000
End	.0018	100 G	1,400	80	880
9. Middle	.0005	100 G	200	10	40
End	.0016	100 G	180	20	10
Average	.0012	100 G	4,700	190	3,100

[1] Inside temperatures: 2½ hours, 37.8°C.(100°F.); 6 hours, 52.8°C.(127°F.).

TABLE 5

Samples Taken After 18 Hours in Smokehouse and Subsequently Chilled[1]

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
10. Middle	.0024	100 G	1,400	1,370	1,400
End	.0013	100 G	5,000	200	70
11. Middle	.0006	100 G	180	0	0
End	.0019	100 G	450	10	0
12. Middle	.0010	100 G	910	0	800
End	.0015	100 G	650	20	0
Average	.0014	100 G	1,400	270	450

[1] Inside temperature, 52.8°C.(127°F.).

TABLE 6

Samples Taken in Smokehouse at End of 18 Hours[1]

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
13. Middle	.0030	100 G	70	10	0
End	.0015	100 G	1,400	0	1,300
14. Middle	.0024	100 G	70	0	0
End	.0024	100 G	13,000	700	60
15. Middle	.0025	100 G	2,700	....	1,600
End	.0024	100 G	230	10	70
Average	.0024	100 G	2.800	120	500

[1] Inside temperature, 52.8°C.(127°F.).

TABLE 7

Samples Taken After 18 Hours' Smoke, and Six Hours' Hanging in Smokehouse

Sample place	Per cent nitrite	Color fixation	Aerobic bacteria on nutrient agar incubated at 20°C.(68°F.) for 72 hours		
			Total count	Oxidizers	H_2S formers
16. Middle	.0006	100 G	2,000	0	2,000
End	.0006	....	140	0	0
17. Middle	.0005	100 G	50	20	0
End	.0010	90 G	900	60	0
18. Middle	.0011	100 G	240	0	0
End	.0013	100 G	140	0	0
Average	.00085	98 G	580	13	330

aliphatic diazo reaction between the residual nitrite and the aliphatic amino group of the protein and its split products, as well as a breaking down of the alpha hydroxy acids which might result in carbon dioxide, etc.

The chemical reaction of the destruction of nitrite may be depicted as follows: (Cured meat is acid pH 5.9 to 6.1)

$$R\left\langle\begin{matrix}CH\,NH\\ CO\,OH\end{matrix}\right. + H\,NO_2 \rightarrow R\left\langle\begin{matrix}CH\text{-}OH\\ CO\,OH\end{matrix}\right. + N_2 + H_2O$$

$$\downarrow$$

$$R\ \ CH\text{-}OH + CO_2$$

Temperature, not time, is considered an important factor for the destruction of nitrite in meat.

Van Slyke (1911) devised a method for quantitative determination of aliphatic amino groups based upon the fact that aliphatic amino groups react with nitrous acid according to the equation:

$$R \cdot NH_2 + H\,NO_2 = R\,OH + H_2O + N_2.$$

He also utilized the oxygen uptake properties of nitrous acid in freeing his apparatus of atmospheric oxygen. Nitrous acid in solution decomposes spontaneously with formation of nitric acid:

$$2\,H\,NO_2 \rightarrow H\,NO_3 + NO.$$

Plimmer (1924) has studied the reaction of nitrous acid upon the hexone bases.

It is our belief that nitrites in cured meats undergoing smoking or heating are destroyed both by the reactions with the amino groups of the protein and by oxidation of the nitrous acid especially on the surface areas of the meat.

There is a very short "incubation period" for bacterial growth—mesophiles, 10 to 43.3°C. (50 to 110°F.)—and the bacterial population does not increase during smoke. In fact, the smoking operation reduces the total bacterial count to very low figures. We must produce bellies out of the box, however, with a very low count of oxidizers or sulphide formers, owing to the fact that color-destroying or color-impairing enzymes and sulphides are not destroyed in the smoke or by the heat in the smokehouse.

In curing, smoking, and processing of smoked meats consideration must be given to three kinds of microorganisms classified according to their thermal growth requirements. These forms are *psychrophiles*, which grow at low temperatures, —2.2 to 7.2°C. (28 to 45°F.); *mesophiles*, the microbes growing between 10 and 40.6°C. (50 and 105°F.); and *thermophiles*, the bacteria growing at temperatures ranging from 43.3 to 65.6°C. (110 to 150°F.).

Many of the bacteria observed during the curing operations are psychrophiles. These bacteria obviously cannot grow during the smoking operations.

The mesophiles will grow in a zone which we call the "incubation zone," i.e., 18.3 to 40.6°C. (65 to 105°F.). When we follow good smoking practices, we do not hold a cured product in this incubation zone longer

than six to eight hours. We open the smokehouse at a temperature which will heat the cured product and "bring it through the zone." According to schedules which are commonly followed in the packing industry, sweet pickle hams are either smoked for 24, 48, or 72 hours, at various temperatures (inside of the hams). These temperatures may be 46.1 to 48.9C. (115 to 120° F.), and some domestic hams are heated to an inside temperature of 58.3°C. (137°F.). Ready-to-eat hams and shoulders are cooked in the smokehouse.

Thermophilic bacteria rarely cause trouble in the smokehouse operations, but on occasion will grow in heavy bologna containing cereal or milk powder if salt (NaCl) is not present in sufficient amounts (over 3.5 per cent) or nitrate is not present in "permissible" amounts.

The constituents of smoke appear to penetrate both natural and artificial casings. Under ordinary conditions the principal foods smoked in this country are hams, shoulders, cured beef hams (dried beef), bacon, frankfurters, table-ready meats like luncheon meats, tongue, liver cheese, braunschweiger, meat loaf, dry sausages, many canned items, etc. Other foods smoked are fish, cheese, cured leg-of-lamb and mutton, turkey, oysters, etc.

The residual effect of the smoke on bacteria is marked, and most smoked, cured products show greatly increased keeping times when compared with unsmoked, cured products. The resistance to mold growth is not so marked, however. Molds and their spores are often very resistant to germicides and antiseptics. The concentration of antiseptics required to inhibit molding usually increases rapidly as the humidity of the air increases. For instance, Orla-Jensen (1939) has made use of the tolerance of molds to formalin to keep pure cultures of *Penicillium roquefortii* on bread substrates containing one per cent formalin. The residual aldehydes from smoke are not especially effective as mycostats. Likewise, the curing agents, notably salt (NaCl), are not effective in hindering molding. There are many strains of obligate halophilic molds, usually divergent forms of brown molds, showing all degrees of salt tolerance. Some are psychrophiles, but most of the strains are mesophiles. Some resemble Torula, others Hemispora, Oospora, or Sporendonema. *Aspergillus candidus* will grow on smoked meats and produce small reddish patches. None of these molds produces substances deleterious to health.

SUMMARY AND CONCLUSIONS

The smoking of meats is an ancient practice, and the modern methods of commercial smoking of cured meats have shown many desirable effects to be gained by proper smoking of these foods. There is a drying effect on the meat which increases durability. Desirable organoleptic properties are imparted to cured meats through hardwood smoking. The constituents of hardwood smoke are not the same as formed from destructive distillation of wood. "Liquid smoke" is not used in commercial meat establishments, and the Federal meat inspection law prohibits use of any "liquid-smoke" preparation.

Proper smoking brings out color inside the cured pieces and impregnates the outside portions of the cured meat with constituents of smoke

which serve as antiseptics and germicides. Bacteria, especially those on the surface, are reduced to very low numbers during the smoking process. Some tendering of the cured meat results from the effect of heat and salt. Nitrite tends to be reduced. A glossy appearance is imparted to the meat if the smoking is properly done. This glossy substance is due to the resins deposited from the condensation of aldehydes and phenols. The small quantity of formaldehyde and phenols in the smoke is often due to the interaction of these two classes of compounds to form the glossy resins.

The constituents of hardwood smoke and their concentrations in the smokehouse are discussed. Some of the constituents of hardwood smoke exert a bactericidal action on nonsporeforming bacteria. A residual antiseptic effect is observed in smoked meats. An antioxidant action in bacon fat owing to these constituents of smoke is observed.

Good smoking practice teaches that most cured meats should not be held in the temperature zone of 18.3 to 40.6°C. (65 to 105°F.) for a longer period than eight hours. This "incubation zone" should be passed as soon as possible without "heat-shelling" the product. The product is, of course, held in the smokehouse for various lengths of time depending upon the nature of the food.

The nonsporing psychrophiles which are active during the curing of bacon are largely destroyed along with the nonsporing mesophiles during the smoking process. Thermophiles, if not inhibited by three and one-half per cent of sodium chloride in cured sausage items containing extenders, such as large bologna with soya grits, may produce gas and spoil the food.

Molds will grow in many smoked meats because of their great resistance to salt and the smoke constituents residual on the meat. There are many strains of obligate, halophilic molds which complicate the preservation of smoked, cured meats.

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EFFECT OF EXTREMELY LOW RATES OF HEAT PENETRATION ON TENDERING OF BEEF[1]

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In several laboratories—Cline, Trowbridge, Foster, and Fry (1930); Kansas Station (1935-36); Latzke (1930)—evidence had been accumulating that low cooking temperature is an important factor in making tough meat tender, but Cover (1937), using different cuts and kinds of meat, reported erratic behavior in tenderness-response to high and low oven temperatures of 225 and 125°C. (437 and 257°F.). The explanation offered was that the difference in cooking time (indicative of the rate of heat penetration) seemed to have more influence on tenderness than did oven temperature. Skewers were found to increase the rate of heat penetration and to make the meat tougher, Cover (1941). The effect on tenderness in none of these studies, however, was of sufficient magnitude to produce uniformly tender roasts in even one cut, but the findings led to the assumption that the effect on tenderness might be accentuated by still slower cooking. One logical way to accomplish this purpose seemed to be by using an extremely low oven temperature.

EXPERIMENTAL PROCEDURE

Paired standing-rib and arm-bone chuck roasts were cooked well-done, and bottom-round roasts were cooked both rare and well-done at oven temperatures of 125 and 80°C. An oven temperature of 80°C. (176°F.) is extremely low, about that of water just beginning to simmer. The roasts cooked in the 125°C. oven served as a normal standard to which the roasts cooked at 80°C. could be compared.

Well-done beef has a characteristic uniform gray color when cut. At an oven temperature of 125°C. or above, it was obtained at an internal temperature of 80°C. Preliminary trials indicated that an internal temperature of 70°C. (158°F.) was sufficient to produce well-done meat in the 80°C. oven. Accordingly, one roast of each pair was cooked to a roast temperature of 70°C. in an oven maintained at 80°C. and the other to a roast temperature of 80°C. in an oven maintained at 125°C.

Rare beef has a characteristic pink color when cut. At an oven temperature of 125°C. or above, it was obtained in bottom-round roasts at an internal temperature of 63°C. (145.4°F.). Preliminary tests with bottom-round roasts cooked in the 80°C. oven showed that an internal temperature as low as 58 or 59°C. (136.4 or 138.2°F.) was necessary for the roasts to be as rare as those cooked to 63°C. in the 125°C. oven. These temperatures, therefore, were used with paired bottom-round roasts in the rare series. The rare bottom-round roasts, like the rare arm-bone chuck roasts in the

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previous study (1937), showed no rise in internal temperature after removal from the oven.

The method of cutting the rib and chuck roasts was the same as previously reported by Cover (1937). The bottom-round roasts were fashioned by cutting at a distance of three and one-half inches from the edge where the round had been severed from the rump. After making the cut through the entire round, the biceps femoris and semitendinosus in the slice were removed in one piece and tied.

Tests for tenderness were made by the paired-eating method, Cover (1940), a five-point scale of weighted adjectives, and the Warner-Bratzler shearing device. The muscles tested for tenderness were the longissimus dorsi in the rib, the triceps brachii in the arm-bone chuck, and the biceps femoris in the bottom-round. Samples for testing were obtained as follows: A one-half-inch slice was cut from the center of each roast and used for subjective tests, as reported previously (1940). Cores for shearing tests were taken from the two outside slices. Only one shear was made on each core and it was made from the side next to that used for subjective tests. Since the cores were taken from the area which corresponded to that used for subjective tests, the samples for subjective and shearing tests were thought to be comparable.

RESULTS AND DISCUSSION

Total cooking losses by the two methods were about the same for paired roasts (Table 1). In view of the large differences in cooking time between

TABLE 1

Cooking Data of Paired Roasts

Cut	Temperature of oven	Weight of roast	Time in oven	Cooking losses		
				Volatile	Fat in drippings	Total [1]
WELL DONE	°C	lb.	hr.	pct.	pct.	pct.
Rib	80	6.7	21.0	13.1	1.0	14.5
	125	6.5	5.1	12.5	5.4	19.4
Chuck	80	6.4	43.3	30.8	1.5	32.9
	125	6.3	8.4	28.1	4.2	34.0
Round	80	3.7	29.8	30.5	1.1	32.7
	125	3.7	6.3	28.8	1.6	32.6
RARE						
Round	80	3.4	8.6	11.6	....[2]	12.4
	125	3.3	2.8	11.4	....[2]	13.7

[1] Meat juices in the drippings are included in total cooking losses. [2] Fat in drippings was too small to measure.

paired roasts, it is remarkable that their volatile losses (largely water) were so nearly alike.

For all of the roasts cooked at an oven temperature of 80°C. the time required for the heat to penetrate into them was much longer than for their pair mates cooked at 125°C. (Table 1). The roasts in which the rate of heat penetration was so much slower were also more tender by all of the measures used for testing tenderness (Table 2).

The rare roasts in this study required about three times as long in the 80°C. oven as in the 125°C. oven and were more tender, but in previous studies by Cover (1937), using oven temperatures of 125 and 225°C., there had been no difference in tenderness between pair mates cooked rare. If the lowest temperature for the change of collagen into gelatin is 58°C., as Wöhlisch (1932) and Wöhlisch and de Rochemont (1926) implied, this change could not be responsible for the difference in tenderness between the rare roasts of the present study because the final internal temperature of the more tender ones was only 58°C. However, unpublished studies by McLaren (1941) at this station show that tendon powder in contact with water is very slowly soluble at temperatures as low as 45°C. (113°F.). Therefore, collagen cannot be eliminated as a factor involved in the tendering of these roasts.

TABLE 2

Tenderness of Paired Roasts

Cut	Number of paired roasts	Temperature of oven	Average rate of heat penetration	Tenderness: Paired-eating method: Number of judgments	Tenderness: Paired-eating method: Tenderness percentage	Tenderness: Weighted adjectives, average per roast [1]	Tenderness: Mechanical shear
		°C.	°C per hr.				lb.
WELL-DONE							
Rib	14	80	2.9	165	88	4.4	16.5
		125	14.4	......	12	3.6	21.6
Chuck	9	80	1.4	90	98	4.9	7.4
		125	8.6	......	2	4.1	14.4
Round	12	80	2.1	189	99	4.5	10.0
		125	11.6	......	1	2.9	17.2
RARE							
Round	10	80	5.9	171	84	4.0	22.0
		125	20.0	......	16	3.4	25.4

[1] Adjective weightings: very tough, 1.0-1.4; tough, 1.5-2.4; neutral, 2.5-3.4; tender, 3.5-4.4; very tender, 4.5-5.0.

After cooking well-done in the 80°C. oven, all of the chuck roasts were scored "very tender," all of the round roasts at least "tender," but not all of the rib and the rare round roasts were scored as high as "tender" (Table 3). The "very tender" chuck roasts had required 43.3 hours to become well-done (no pink color), the "tender" round roasts 29.8 hours, the "not always tender" rib roasts 21 hours, and the "not always tender" rare round roasts 8.6 hours. Thus roasts which were always tender were obtained when the rate of heat penetration was slow enough so that it required 30 hours or more for the meat to lose its pink color. When the time was much shorter than 30 hours the roasts were not always tender. These observations made from weighted adjectives are borne out by the mechanical shear values (Table 3) which are uniformly low (5.5 to 8.7 pounds) for the well-done chuck roasts cooked at 80°C., are still reasonably low for the well-done bottom-rounds (6.2 to 14.3 pounds), but are considerably higher for the standing-ribs (12.2 to 21.1

TABLE 3

Weighted Adjectives and Mechanical Shear Values for Individual Roasts

Well-done												Rare			
Arm-bone chuck				Bottom-round				Standing-rib				Bottom-round			
Weighted adjectives, average per roast		Mechanical shear		Weighted adjectives, average per roast		Mechanical shear		Weighted adjectives, average per roast		Mechanical shear		Weighted adjectives, average per roast		Mechanical shear	
80°C.	125°C.	80°C.	125°C.	80°C.	125°C.	80°C.	125°C.	80°C.	125°C.	80°C.	125°C.	80°C.	125°C.	80°C.	125°C.
		lb.	lb.			lb.	lb.			lb.	lb.			lb.	lb.
5.0[1]	5.0	5.5	11.5	5.0	3.5	6.3	13.3	5.0	4.7	....	....	4.8	4.2	15.8	20.4
5.0	4.7	5.8	11.8	5.0	3.6	6.2	17.6	5.0	4.2	12.5	18.4	4.8	3.7	13.5	17.1
5.0	4.6	8.7	11.2	4.9	3.1	10.3	16.8	4.9	4.4	....	....	4.6	4.3	17.8	17.1
5.0	4.3	6.4	11.8	4.9	3.4	8.1	17.8	4.9	3.5	....	....	4.3	3.8	17.2	20.0
5.0	4.0	7.4	16.1	4.7	2.6	12.2	23.7	4.9	3.4	12.2	21.5	4.1	3.3	21.5	26.2
4.9	4.3	8.1	15.2	4.7	3.5	9.3	14.6	4.8	4.6	12.4	15.3	3.9	3.6	23.8	27.5
4.8	3.1	7.9	18.1	4.5	2.7	10.8	19.2	4.6	3.7	14.5	24.3	3.9	3.4	....	....
4.7	2.9	8.6	18.7	4.4	2.9	10.0	16.2	4.5	3.1	14.9	20.8	3.4	3.3	30.3	30.4
4.7	3.6	8.1	15.5	4.3	2.4	12.8	16.3	4.4	4.6	18.8	19.4	2.9	2.2	29.7	35.2
....	....	....	....	4.2	2.7	9.9	14.8	4.3	3.0	20.6	27.7	2.8	2.3	28.7	34.3
....	....	....	....	4.0	2.4	9.7	16.3	3.8	3.0	19.9	23.0	....	....	....	....
....	....	....	....	3.9	1.9	14.3	19.8	3.6	3.0	....	....	....	....	....	....
....	....	....	....	....	....	....	....	3.4	3.2	17.6	22.1	....	....	....	....
....	....	....	....	....	....	....	....	3.3	1.9	21.1	23.6	....	....	....	....
Mean 4.9	4.1	7.4	14.4	4.5	2.9	10.0	17.2	4.4	3.6	16.5	21.6	4.0	3.4	22.0	25.4

[1] Adjective weightings: very tough, 1.0-1.4; tough, 1.5-2.4; neutral, 2.5-3.4; tender, 3.5-4.4; very tender, 4.5-5.0.

pounds), and are much higher still for the rare bottom-round roasts (13.5 to 30.3 pounds).

Popular taste favors a roast which has a plump appearance. The well-done roasts cooked at 80°C. were not plump. They were more nearly the shape of the uncooked roast and seemed to be little if any smaller in size than those cooked at 125°C. It was strikingly evident in the roasts cooked at 125°C. that contraction of the connective tissue masses had squeezed the muscle tissue toward the cut surface giving the roasts a plump appearance, while in the roasts cooked at 80°C. this shortening of the connective tissue masses had not taken place. Since Wöhlisch and de Rochemont (1926) reported that thermal shortening of collagen begins at 60°C. (140°F.), shortening would have been expected in well-done roasts had not the change in collagen progressed beyond the point where this could take place. Yet not all of the rib and well-done bottom-round roasts were "very tender" as would have been expected had all of the collagen been changed completely and if collagen were the only factor influencing tenderness.

Since connective tissue was thought to be a factor of particular importance in influencing the original toughness of the bottom-round roasts, the appearance of the connective tissue after cooking was observed closely. In the well-done roasts cooked at an oven temperature of 80°C. the coarse connective tissue appeared to be completely changed from its hard and tough state to a moist, viscous mass which was without resistance to either the knife or teeth. It seemed to be responsible for most of the juiciness in these roasts. (The roasts were judged warm.) In the pair mates cooked at 125°C. the pieces of connective tissue seemed to be softened considerably but had not disintegrated, were still plainly visible when the roasts were cut, and offered considerable resistance to mastication. As the roasts were in the 80°C. oven more than 20 hours after an internal temperature of 57°C. was reached and the moisture loss was not large, perhaps the water of hydration was released slowly enough from the meat proteins during the extremely slow coagulation so that it was used efficiently for converting the collagen into gelatin. Since satisfactory quantitative chemical tests for collagen in cooked meat have not been developed, there was no adequate measure of the chemical changes which seem to have taken place. Weighted adjectives and shearing tests agree, however, that these roasts were not always "very tender." Apparently, this must have been caused by a factor other than connective tissue.

Lehmann (1907), though unable to find a satisfactory explanation of his results with tenderness, suggested that two opposing forces were involved: 1. The contraction of the muscle on cooking, which presses out water and causes the muscle to become denser, ought to increase its toughness. 2. The change of collagenous tissue to gelatin should increase tenderness. In previous meat-cookery experiments there has been no reason to think that these two forces might not be the correct causes of the changes observed, but in the roasts of the present study which required approximately 40 hours to lose their pink color (arm-bone chuck), the meat fibers were so tender that they could be broken between the tongue and the roof of the mouth. All of them were judged "very tender." Their

low shearing strengths of 5.5 to 8.7 pounds also indicate very tender meat. Apparently, the conditions under which they were cooked had brought about changes in the muscle fibers themselves, different from those observed in usual cooking, with the result that the fibers did not furnish a toughness to oppose the tenderness produced in the connective tissue. Thus the opposing forces mentioned by Lehmann were not found in this cut.

In view of this reversal of the usual effect of heat on the tenderness of muscle fibers it should be noted that the muscle fibers from the 40-hour roasts appeared dry in the mouth but very tender while those from their six-hour pair mates cooked at 125°C. appeared juicier but less tender. Whether or not these observations are related to the true moisture content of the fibers themselves was not determined, but in these roasts apparent dryness was associated with unusual tenderness. The question may be raised whether moisture may not in some way play as great a part in the tenderness of muscle fibers as it appears to do in connective tissue.

Records of appearance, flavor, and juiciness were not made by the judges because it seemed best for them to concentrate their attention on tenderness. But observations made by the author during cooking and sampling may be of interest in discouraging the immediate recommendation of such an extremely low oven temperature method for home use. In the roasts cooked at 80°C. the fat on the outside had darkened slightly but had not really browned. It looked somewhat greasy. The exposed lean did not have the grey appearance of water-cooked meat nor yet the rich brown color of seared meat but rather the brown or dark appearance more similar to dried meat. When eaten, the meat inside the roast appeared to be less juicy and to have less flavor than that cooked at 125°C. Slicing was occasionally a problem because the meat tended to flake off somewhat like fish during cutting and it was not always possible to obtain smooth slices. As for texture, the dry, granular feel of the broken meat fibers in the mouth was described as "mealy"—a most unusual adjective to apply to meat yet appropriate because of the unusual lack of cohesiveness in the meat fibers themselves as well as in the material which binds meat fibers together. In contrast to these obvious objections it was noted that after the tests were completed the meat remaining from the roasts cooked at 80°C. found an even readier sale than did that from the roasts cooked at 125°C. The reason given was its greater tenderness.

SUMMARY

Paired roasts from three cuts were cooked well done and one cut rare at oven temperatures of 80 and 125°C.(176 and 257°F.). They were tested for tenderness by the paired-eating method, weighted adjectives, and the Warner-Bratzler shear.

Roasts which were always tender were obtained when the rate of heat penetration was slow enough so that it required 30 hours or more for them to lose their pink color. With less time the roasts were not always tender.

Well-done roasts from the 80°C. oven were not plump although their internal temperatures had reached 70°C.(158°F.), high enough for the heat to have contracted the collagen which they contained. This is taken as

evidence that in these roasts the conversion of collagen had progressed beyond the point where contraction could take place.

The large amount of connective tissue in well-done bottom-round roasts cooked at 80°C. appeared to be completely changed from its hard and tough state to a moist, viscous mass which, while warm, was without resistance to either the knife or the teeth. Since the moisture loss from these roasts was quite moderate in amount and the coagulation time was very long, it is suggested that the water of hydration was released slowly enough from the meat proteins so that it was used effectively for converting collagen into gelatin.

The muscle fibers in the well-done arm-bone chuck roasts, which required 40 hours to lose their pink color, were so very tender that the judges described them as "mealy," a finding quite different from the usual conception that the muscle fiber during cooking becomes denser and tougher.

It appears that there are two structures in meat which contribute to its toughness, muscle fiber and connective tissue. Both may be made "very tender" by cooking if the cooking is slow enough so that 40 hours are required for the meat to lose its pink color. The chemical factor causing tendering in connective tissue appears to be the change from collagen into gelatin, but the one causing tendering in muscle fibers was not identified.

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CHANGES OCCURRING IN FRUIT JUICES DURING STORAGE[1]

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A number of pleasing fruit-juice beverages may be prepared from the various small fruits which should find a ready market if properly handled. Unfortunately, as in many other foods, a slow deterioration in quality occurs during storage which cannot be attributed to the presence of microorganisms, enzymes, or to reaction between the juice and the container.

Little is known in regard to the changes occurring during storage. Mottern and von Loesecke (1933) believed oxygen to be an important factor in the deterioration of orange juice and devised a deaeration apparatus for removing air from the juice. A number of papers have since appeared discussing the relationship of oxygen to quality in orange juice. These are reviewed in recent papers by Hamburger and Joslyn (1941) and Moore, Esselen, and Fellers (1942). Hamburger and Joslyn (1941) suggest that the darkening occurs after the ascorbic acid is in the dehydro form and when no readily oxidizable substances are left in the juice. They further state that the principal reducing agents are ascorbic acid and flavanols. Moore, Esselen, and Fellers (1942) observed changes in orange juice which could be correlated with loss of ascorbic acid.

Tressler and Pederson (1936) observed certain changes occurring in grape juice that could be partially overcome by deaeration and pasteurization at temperatures lower than ordinarily used, by filling containers full with hot juice to exclude air, and by low-temperature storage. In apple juice, darkening of color, change in flavor, and excessive sedimentation have occurred simultaneously—Pederson and Tressler (1938) and Tressler, Pederson, and Beattie (1943). Pederson, Beattie, and Beavens (1941) also observed the relationship of the temperature of storage to changes in flavor, color, and sometimes ascorbic acid content.

Kirk and Tressler (1941) studied the losses of ascorbic acid in strawberry juice as well as other highly pigmented juices. Preparation of fruit, pressing, or any factor increasing the oxygen content, caused a loss of ascorbic acid. Charley (1941) observed pronounced losses of ascorbic acid in strawberry juice during storage with marked change in flavor.

Although all fruit juices or blends show some deterioration during storage, the changes are most marked in strawberry juice. When properly prepared, the color of strawberry juice is bright and readily compared; the ascorbic acid content is fairly high and therefore the juice lends itself to study. The color changes from bright clear red to dull red to brown. The fresh strawberry flavor changes to a flat almost oily one. This may be partially masked in certain products by addition of sugar and acid. Some clouding may occur. These changes have been observed in other juices and

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blends, such as grape, currant, rhubarb, raspberry juices, apple-raspberry, apple-strawberry, and similar blends. Since the red pigments of strawberry, currant, and raspberry are readily reducible and the ascorbic acid is readily oxidizable, the interrelation between the two seems a possibility.

PREPARATION AND ANALYSIS OF JUICE

Juices were prepared from currants, nine varieties of raspberries, and two varieties of strawberries. The juices were usually prepared from fruit which had been frozen with 10 to 12 per cent of sugar. The frozen fruit was thawed and pressed in an hydraulic press while still cold, with the exception of currants which were heated to 65°C.(149°F.) before pressing. The expressed juices were immediately deaerated at six to 12 cm. of mercury pressure in a deaerator of the type described by Mottern and von Loesecke (1933) and Pederson and Tressler (1938). The deaerated juices were pasteurized by heating in a stainless-steel kettle. Heated bottles were filled full with juice at the pasteurizing temperature. The temperature of pasteurization varied with each lot. In order to obtain juices of an intensity of color that could be measured readily in the colorimeter, it was necessary to dilute some of the more highly pigmented juice with definite amounts of water and then filter them to remove suspended material.

Total sugar, reducing sugar, and total acid were determined for each juice before and after storage. In addition, the specific gravity, refractive index, degrees Brix, and pH were obtained. At intervals during storage, color was measured in a "British Drug House" pattern Lovibond colorimeter, and ascorbic acid determined by titration with 2, 6 dichlorophenol-indophenol—Bessey and King (1933) as modified by Mack and Tressler (1937)—or by the electrometric titration method of Kirk and Tressler (1939).

CHANGES IN JUICE DURING STORAGE

Currant: The currant juice had a bright red color when pressed. It was extremely tart, acidity 2.5 per cent as malic acid, hydrogen-ion concentration pH 3.15. The freshly pressed juice contained .44 mgm. of ascorbic acid per gram, but the bottled juice contained only .15 mgm. per gram. Juice was bottled at 74, 86, and 93° C.(165, 187, and 200° F.). Bottles of juice were stored at 1, 7, 21, and 32° C.(33.8, 44.6, 69.8, and 89.6°F.) in the dark and at 21°C. in the light.

During storage a progressive darkening in color at higher temperatures was observed. Analysis at the end of three months showed little change in acidity or ascorbic acid content but a considerable change in color (Table 1). At the higher temperatures, a marked loss in red color and an increase in yellow had occurred. The relation of red to yellow color (red units divided by yellow units) varied from 4.75 for juice at 1°C. to .70 for juice stored at 32°C. (Table 1). Flavor changes occurred at the higher temperature simultaneously with color changes.

Raspberry: The nine varieties of raspberries yielded from 62 to 70 per cent of juice by weight. They were all bright red to deep clear purple-red, dependent upon variety. They were bottled at 82 to 85°C. (180 to 185°F.). Bottles of juice were stored at 1, 21, and 32°C. in the dark and at 21°C. in the light. Analyses were made after two and three months of storage.

During storage at higher temperatures, a progressive browning of the juice occurred and sediment was deposited. After two months, the flavor of all except that stored at 1°C. was poor to fair. After three months' storage these changes were intensified. Those juices stored at 1°C. were very good. Little change in ascorbic acid occurred (Table 2). On the

TABLE 1

Effect of Storage Conditions Upon Color and Ascorbic Acid Content of Currant Juices After Three Months

Storage temperature	Acidity [1] as malic	Ascorbic acid [2]	Lovibond relative color, dilution 1-1		
			Red	Yellow	Red/Yellow
°C.	pct.	mgm./gm.			
1	2.53	.13	11.4	2.4	4.75
7	2.53	.13	9.5	2.3	4.12
21 [3]	2.50	.12	5.1	3.5	1.45
21	2.74	.12	5.3	3.5	1.51
32	2.55	.11	4.2	6.0	0.70

[1] Hydrogen-ion concentration pH 3.15. [2] Ascorbic acid content when bottled, .15 mgm. per gm. [3] Stored in sunlight.

TABLE 2

Effect of Storage Conditions Upon Raspberry Juices

Temperature of storage	Indian Summer [1]				Viking [1]			
	Ascorbic acid	Lovibond relative color 3-1			Ascorbic acid	Lovibond relative color 1-1		
°C.	mgm./gm.	*Red*	*Yellow*	*R/Y*	mgm./gm.	*Red*	*Yellow*	*R/Y*
1	.16	27.7	8.0	3.5	.18	23.0	9.0	2.6
21	.18	28.8	8.0	3.6	.17	22.3	5.1	4.4
21 [3]	.16	24.0	5.4	4.4	.18	15.8	3.3	4.8
32	.17	23.1	4.0	5.8	.18	19.9	4.0	5.0
Temperature of storage	**Latham [2]**				**Newburg [2]**			
	Ascorbic acid	Lovibond relative color 1-1			Ascorbic acid	Lovibond relative color 1-1		
°C.	mgm./gm.	*Red*	*Yellow*	*R/Y*	mgm./gm.	*Red*	*Yellow*	*R/Y*
1	.14	27.4	9.3	2.9	.24	19.7	7.1	2.7
21	.14	26.2	9.1	2.9	.23	17.4	6.1	2.8
21 [3]	.14	20.3	6.0	3.4	.23	12.6	3.3	3.8
32	.14	17.1	4.1	4.2	.20	9.2	3.3	2.8

[1] Stored two months. [2] Stored three months. [3] Stored in sunlight.

other hand, the change in relative color was great, particularly after three months. The red as well as the yellow color of Newburg juice stored at 32°C. was less than half as intense as juice stored at 1°C. The intensity of yellow color was reduced more than the red in all cases.

Strawberry—Effect of Temperature of Pasteurization: Culver strawberries produced a bright red, clear juice which was bottled at 74, 79, 85, and 93°C. (165, 175, 185, and 200°F.) and stored at 1, 7, 21, and 32°C.

After two and three months, juices stored at 1 and 7°C. were still bright red in color and had good flavor. Those stored at 21°C. had yellowed but were still fair, but those at 32°C. were dark brown and poor in

flavor. Little if any difference could be noted between samples bottled at the various temperatures, although a variation owing to concentration by heating at 93°C. had occurred. This caused an acidity change from .98 to 1.01 per cent acid as citric. These percentages as well as the Brix reading, 20.5 to 21.0°, and hydrogen-ion concentration, pH 3.40, did not change during storage.

Although little difference was noted between juices bottled at different temperatures, a greater relative loss in color as well as ascorbic acid occurred in this juice as compared with the currant or raspberry juices (Table 3). In three months at 32°C. the ascorbic acid had been reduced from .35 to .19 mgm. per gram and the red color to 5.0 units of red but the yellow had increased to 12.2 units. These changes caused a variation in red/yellow index from 1.4 at 1°C. to .4 at 32°C., quite contrary to results obtained with raspberry juice. The stronger color of juice bottled at 93°C. seems to be more intense than may be accounted for by the concentration resulting from heating to the higher temperature.

TABLE 3

Ascorbic Acid and Relative Color of Strawberry Juices Bottled at 74 and 93°C. (165 and 200°F.) and Stored Three Months at Different Temperatures

Temperature of storage	Bottled at 74°C.				Bottled at 93°C.			
	Ascorbic [1] acid	Lovibond relative color			Ascorbic [1] acid	Lovibond relative color		
		Red	Yellow	R/Y		Red	Yellow	R/Y
°C.	*mgm./gm.*				*mgm./gm.*			
1	.27	9.0	6.6	1.4	.25	9.7	7.0	1.4
7	.26	7.7	6.0	1.3	.27	8.7	6.5	1.3
21	.20	4.2	6.9	0.6	.22	5.2	8.0	0.6
21[2]	.19	4.5	7.4	0.6	.22	4.8	7.6	0.6
32	.19	5.0	12.2	0.4	.21	5.3	12.6	0.4

[1] Ascorbic acid freshly bottled, .35 mgm. per gm. [2] Stored in sunlight

Strawberry—Effect of Head Space in Bottle: Culver strawberry juice prepared as above and pasteurized at 85 to 88°C. (185 to 190°F.) was packed in two ways. One lot of bottles was filled full of juice at the temperature of pasteurization, and in the other lot about one inch of head space was allowed. The same storage conditions were used. The juices were examined after two and four weeks.

Changes similar to those described above occurred during storage, but they were far more marked in bottles having greater head space (Table 4). The loss in ascorbic acid in these bottles was considerably greater than in filled bottles and the change in color was more pronounced. The increase in yellow color at raised temperatures noted before (Table 3) was not observed in this series. This may be accounted for partially by the shorter period of storage.

Strawberry—Effect of Method of Pressing: Freshly picked Dresden strawberries were divided into three lots. The first lot (A) was pressed cold after washing, slicing, and adding 10 per cent of sugar; the second (B) was pressed after heating the washed, sliced, and sugared berries to 60°C. (140°F.); and the third (C) was frozen at —18°C (—.4°F.) after washing, slicing, and sugaring, and then thawed after two days and pressed

cold. All juices were deaerated immediately after extraction, and pasteurized and bottled at 85°C.(185°F.); bottles were stored at 1, 21, and 32°C. (33.8, 69.8, and 89.6°F.).

The changes occurring during storage were similar to those discussed previously. Darkening of color and development of the objectionable, somewhat oily flavor occurred very early at 21 and 32°C.

Other than changes in color and ascorbic acid content, very little change in chemical composition was noted. The acidity did not change appreciably after nine months' storage at 21°C., or 17 months' at 1°C. (Table 5). A

TABLE 4

Effect of Air in Bottles Upon Strawberry Juices Stored Under Varying Conditions

Temperature of storage	Time in weeks	Bottles full				Bottles with air space			
		Ascorbic acid	Lovibond relative color			Ascorbic acid	Lovibond relative color		
			Red	Yellow	R/Y		Red	Yellow	R/Y
°C.		mgm./gm.				mgm./gm.			
1	0	.39		....	....	....	....	....	....
....	4	.34	8.1	5.4	1.50	....	....	....	....
7	4	.33	7.7	5.1	1.51	.12	7.1	5.1	1.49
21	4	.24	6.2	4.2	1.47	....	....	....	....
21[1]	2	.30	6.8	4.4	1.54	.17	4.7	3.8	1.23
....	4	.20	5.1	3.6	1.42	.05	3.4	3.2	1.06
32	2	.28	6.2	5.0	1.24	.12	4.8	5.2	0.92
....	4	.21	4.5	4.2	1.07	....	....	....	....

[1] Stored in sunlight.

TABLE 5

Chemical Composition of Strawberry Juices

Treatment	Specific gravity	Total sugar	Reducing sugar	Degrees Brix	Viscosity relative time	Acidity as citric acid	Hydrogen-ion concentration
		pct.	pct.			pct.	pH
A—Cold pressed	1.071	16.6	4.4	17.0	6.85	.77	3.39
B—Hot pressed	1.067	15.0	3.7	16.5	8.18	.78	3.39
C—Frozen and pressed	1.084	17.6	7.2	18.5	8.68	.79	3.42
D—Culver cold pressed	1.073	....	....	17.5	....	.95	3.61

slight rise in hydrogen-ion concentration from pH 3.33 to 3.39 and 3.38 to 3.42 was recorded but its significance is doubtful. It may be noted that the three juices vary as to viscosity (Table 5) and color (Table 6). During storage, a marked decrease in intensity of both red and yellow occurred. The changes progressed more rapidly at the higher temperatures (Table 6), the change at 32°C. in one month being almost as great as the change at 1°C. in 17 months.

The observed color of the juice stored for one month at 32°C. was brown; that stored at 1°C. for 17 months was still light red. The flavor of the latter juice was good while that of the former was poor. This is not in agreement with measured changes in that the color of the samples stored at 32°C. was worse than measurements show. The difference between observed and measured color may be due to the fact that filtering

is essential to measuring color. Filtration removes the brown suspended solids leaving the juice light red in color, and therefore juices stored at 32°C. are actually poorer than the figures show (Table 6).

The loss in ascorbic acid was about the same in 17 months at 1°C. as in one month at 32°C.

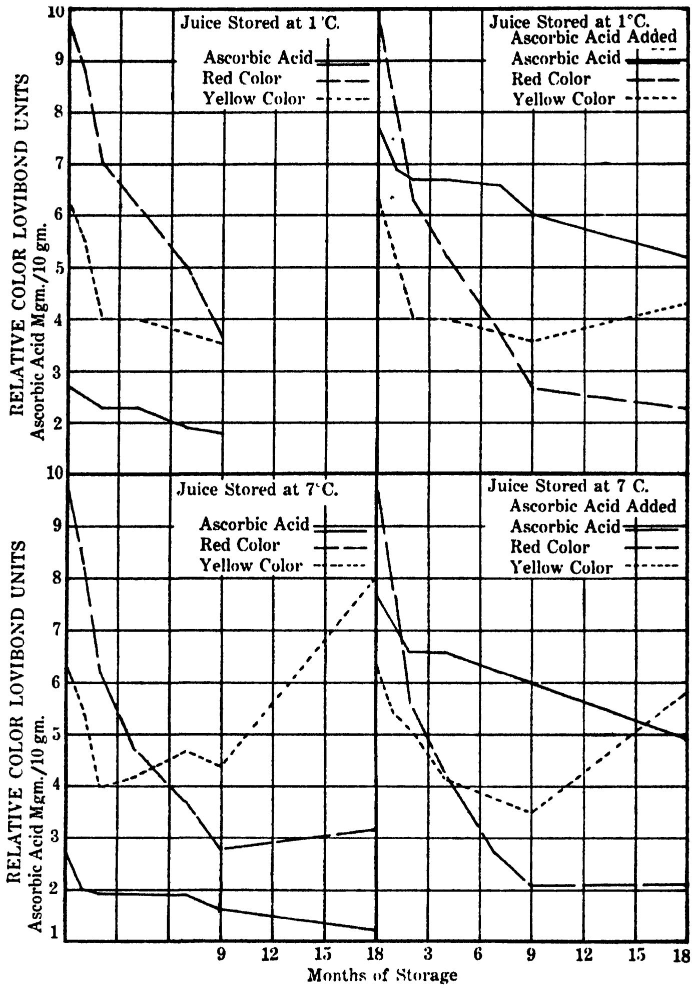

FIG. 1a. Effect of temperature and of ascorbic acid upon color and ascorbic acid content of strawberry juice stored at 1 and 7°C. (33.8 and 44.6°F.).

Strawberry—Effect of Added Ascorbic and Isoascorbic Acid: Frozen Culver strawberries were thawed, pressed, and the juice deaerated and divided into four lots. The first portion of juice was pasteurized and bottled at 85°C. (185°F.). To the second was added 50 mgm. of ascorbic acid per 100 c.c. of juice; to the third, 25 mgm. of ascorbic acid and 25 mgm. of isoascorbic acid; and to the fourth, 50 mgm. of isoascorbic acid before pasteurizing and bottling. Bottles of juice were stored in the dark at 1, 7, 21, and 32°C.

TABLE 6

Effect of Time and Temperature Upon Ascorbic Acid and Color of Dresden Strawberry Juice

Temperature of storage	Time of storage	A—Cold pressed strawberries				B—Hot pressed strawberries				C—Pressed from frozen strawberries			
		Ascorbic acid	Lovibond relative color			Ascorbic acid	Lovibond relative color			Ascorbic acid	Lovibond relative color		
			Red	Yellow	R/Y		Red	Yellow	R/Y		Red	Yellow	R/Y
°C.	mo.	mgm /gm.				mgm /gm				mgm /gm			
1	0	.49	7.3	4.7	1.5	.46	8.9	7.0	1.3	.43	7.0	4.2	1.6
	1	.50	6.1	3.9	1.5	.45	8.4	6.0	1.4	.41	6.2	4.0	1.5
	2	.48	5.8	4.1	1.4	.44	8.2	7.1	1.2	.42	6.3	4.3	1.5
	4	.50	5.6	3.9	1.4	.44	6.7	5.5	1.2	.44	5.1	3.3	1.5
	7	.51	4.7	3.0	1.5	.42	6.2	4.2	1.4	.35	4.3	3.0	1.4
	9	.44	4.1	3.2	1.3	.41	5.5	5.0	1.1	.38	3.2	2.2	1.4
	17	.40	3.6	3.0	1.2	.38	4.4	4.2	1.0	.31	3.0	3.0	1.0
21	0	.49	7.3	4.7	1.5	.46	8.9	7.0	1.3	.43	7.0	4.2	1.6
	1	.45	4.6	3.4	1.4	.40	6.6	6.4	1.0	.36	4.5	3.5	1.3
	2	.43	4.0	3.1	1.3	.35	4.5	4.1	1.1	....	3.1	3.0	1.0
	4	.44	3.3	2.7	1.2	.41	4.1	4.5	0.9	.34	2.3	2.8	0.8
	7	.43	....	....	....	.37	....	....	....	.43	....	....	....
	9	.37	2.1	2.5	0.8	.35	3.0	4.8	0.6	.29	2.0	4.0	0.5
32	0	.49	7.3	4.7	1.5	.46	8.9	7.0	1.3	.43	7.0	4.2	1.6
	1	.44	3.6	3.3	1.1	.35	4.6	4.9	0.9	.31	2.8	3.5	0.8
	2	.42	2.7	3.0	0.9	.37	4.0	5.4	0.7	.32	2.3	4.0	0.6

The samples were examined over a longer period so that a more complete picture of the changes could be observed (Figs. 1a and 1b). The observed differences between the juices to which was added ascorbic or isoascorbic acid were so slight that the average of these results was presented. Color and flavor changes occurred during storage, that is, the same browning of color and development of "off" or "oil" flavor at 21

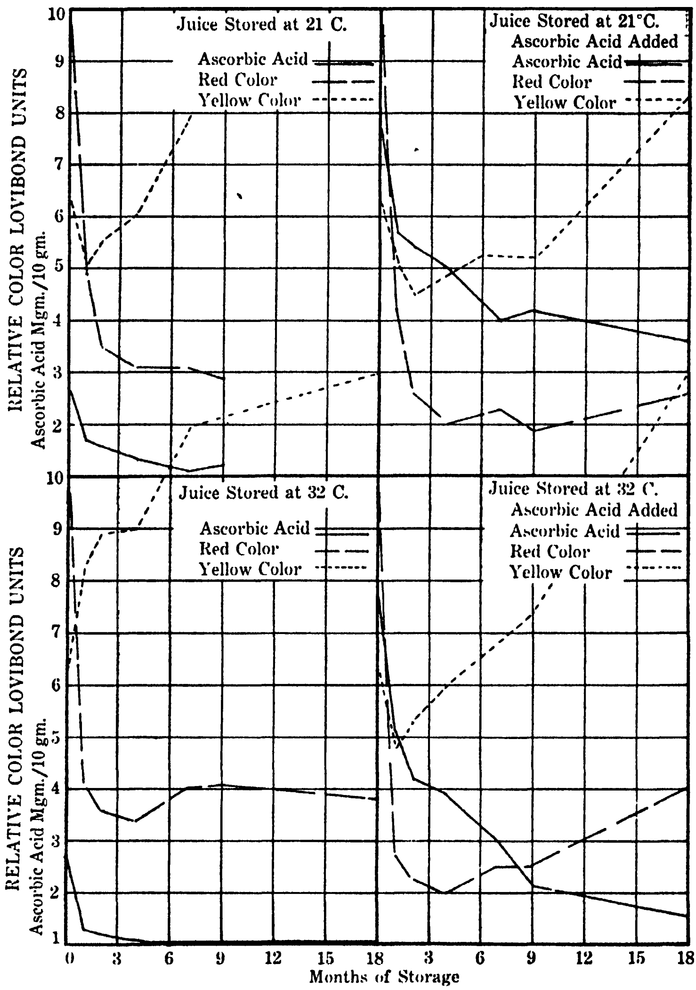

Fig. 1b. Effect of temperature and of ascorbic acid upon color and ascorbic acid content of strawberry juice stored at 21 and 32°C. (69.8 and 89.6°F.).

and 32°C. The chemical composition remained practically the same during storage (Table 5) although the pH of the juices at 7, 21, and 32°C. was slightly higher, pH 3.65. However, changes in ascorbic acid and color were even more marked than in any of the previous tests. The results (Figs. 1a and 1b) show progressive losses in ascorbic acid and in red color. Preliminary losses in yellow color were followed by increases. Although

changes at 1°C. were no more marked after 18 months than in one month at 32°C., the type of change which occurred was not the same in all respects. First it is noted that the red color and ascorbic acid decrease in about the same degree regardless of temperature. The yellow color reverses itself more quickly at the higher temperatures and increases in intensity. At first thought the red/yellow index seems to vary considerably more at 32 than at 1°C., but actually the final results are about the same. A red/yellow index of about .40 is reached in two months at 32°C., in seven months at 21°C., and in 18 months at 7°C. Indications lead one to believe that the 1°C. result would be the same if held for a long enough period of time. However, it should be noted that the results are not the same when ascorbic acid is added.

If a reaction occurred between ascorbic acid and pigments of the juice, then one might obtain a greater reaction if the concentration of one or the other is increased. The curves (Figs. 1a and 1b) show that when ascorbic acid, or its isomer, was added, a more rapid reduction in red and yellow color occurred, the red color was less intense when the rate of destruction levelled off, the increase in yellow following the original decrease was slower, and the amount of ascorbic acid lost was considerably greater.

SUMMARY

Processed strawberry, raspberry, and currant juices contain appreciable amounts of ascorbic acid (vitamin C). The ascorbic acid progressively decreases during storage, the rate of destruction being more rapid at higher storage temperatures.

The red and yellow colors are reduced, the former at about the same rate at which ascorbic acid is destroyed. After the initial loss of yellow color its intensity is increased. Differences in temperature of pasteurization or in method of extraction have shown little effect upon the changes occurring during storage.

The presence of air causes a marked increase in destruction of ascorbic acid and has some effect upon the change in color. Since ascorbic acid is oxidizable and pigments reducible, the results suggest that they may react with each other. Increasing the concentration of ascorbic acid or its isomer increases the rate of change of color as well as the rate of loss of ascorbic acid.

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BUFFERING EFFECT OF FRUIT JUICES [1]

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In studying the various blends of fruit juices—Pederson, Beattie, and Beavens (1941); Beattie and Pederson (1942); Tressler, Pederson, and Beattie (1943); and Pederson and Beattie (1943)—it has been observed that the hydrogen-ion concentration and total acidity are not as closely correlated as might be expected. Since the heat required for sterilizing is dependent upon the hydrogen-ion concentration, it seemed worth while to have more complete knowledge regarding the acidity and buffering effect of the various fruit juices. Newton and Edwards (1931) found that the buffering effect of fruit juices varied with maturity of fruit as well as variety of fruit. Beattie and Pederson (1943) observed a marked variation in buffering effect not only between juices extracted from various vegetables but also between different samples of the same vegetable.

The hydrogen-ion concentration of juices was determined by using a Beckman pH meter with a glass electrode. One hundred cubic centimeter quantities of the juices were titrated with one-tenth normal sodium hydroxide, hydrochloric acid, and tartaric acid solutions. The hydrogen-ion concentration was determined at regular intervals during titration so that standard buffer curves (Figs. 1, 2, and 3) could be drawn. All were titrated to a level of about pH 2.7 to 2.9 with tartaric acid and 1.7 to 1.9 with hydrochloric acid, but were not titrated with sodium hydroxide to level the curve.

The results show a considerable variation between the various juices. The acidity was found not to correlate with the hydrogen-ion concentration as closely as was expected; for example, the 1942 apple juice No. 42 has a lower pH as well as a lower acidity than the 1941 No. 41 juice. After addition of only a small amount of sodium hydroxide the curves cross (Fig. 2). A number of other curves cross, showing lack of correlation between hydrogen-ion concentration and titratable acidity. The most outstanding is the cherry juice curve No. 11 which crosses curves (Figs. 1, 2, 3) No. 1, 6, 7, 9, and 10 on the alkaline side, and No. 2, 3, 4, 5, and 8 when titrated with tartaric acid. From these data it may be noted that the cherry and currant juices are the most highly buffered, while the quince and elderberry are the most poorly buffered. The buffering effect cannot be correlated with ash content (Table 1).

It has been shown by Pederson and Tressler (1938) that fruit juices of high hydrogen-ion concentration may be pasteurized safely at relatively low temperatures. In blending various fruit juices with apple juice, one cannot always depend upon the apple juice to raise the hydrogen-ion concentration sufficiently so that the same pasteurization may be used as that

[1] Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 551, March 18, 1943.

TABLE 1

Comparative Analysis of Fruit Juices

Kind of juice	Hydrogen-ion concentration	Acidity as citric acid			
		At pH 7.0	At pH 8.4	Difference	Ash
	pH	*pct.*	*pct.*	*pct.*	*pct.*
Strawberry juice	3.44	0.560	0.610	.050	.128
Black raspberry juice	3.78	0.560	0.644	.084	.227
Blackberry juice	3.84	0.490	0.560	.070	.218
Elderberry juice	4.27	0.406	0.483	.077	.438
Quince juice	3.63	0.476	0.490	.014	.184
Crabapple juice	3.42	0.680	0.714	.034	.310
Apple juice No. 42	3.48	0.539	0.576	.028	......
Apple juice No. 41	3.69	0.602	0.637	.035	......
Grape juice	3.13	0.826	0.841	.015	.227
Grape juice	3.15	0.889	0.917	.028	.215
Cherry juice	3.50	1.022	1.071	.049	.291
Currant juice	3.19	1.827	1.925	.098	.281

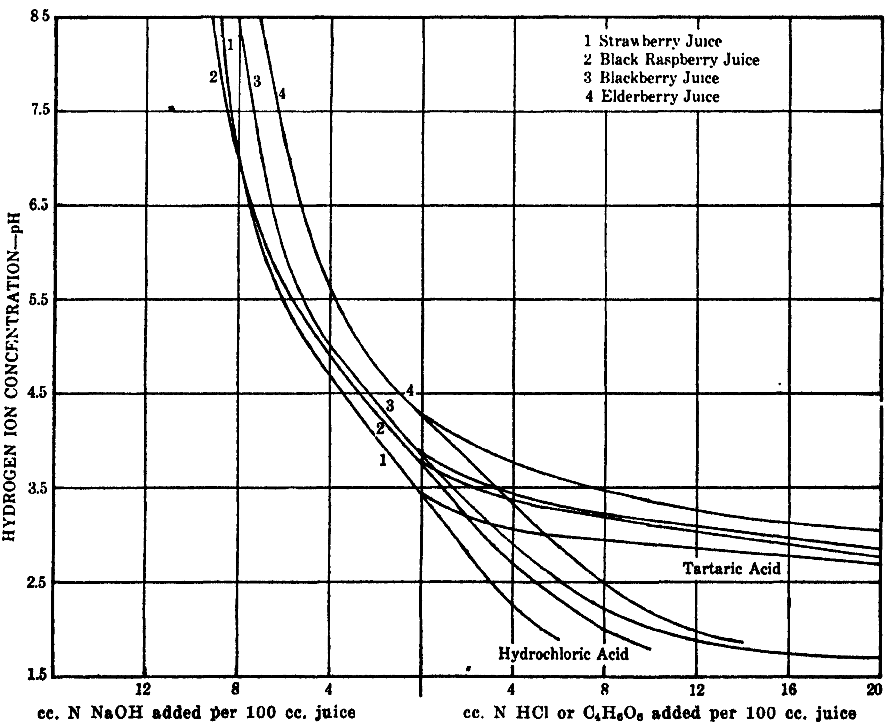

FIG. 1. Comparative buffering effect of strawberry, black raspberry, blackberry, and elderberry juices.

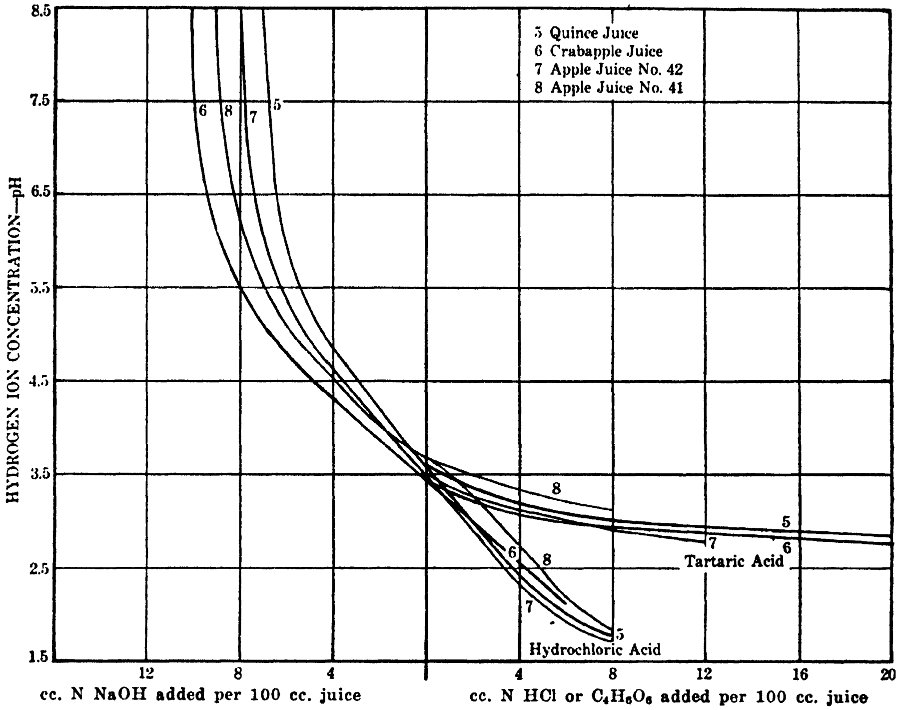

FIG. 2. Comparative buffering effect of quince, crabapple, and apple juices.

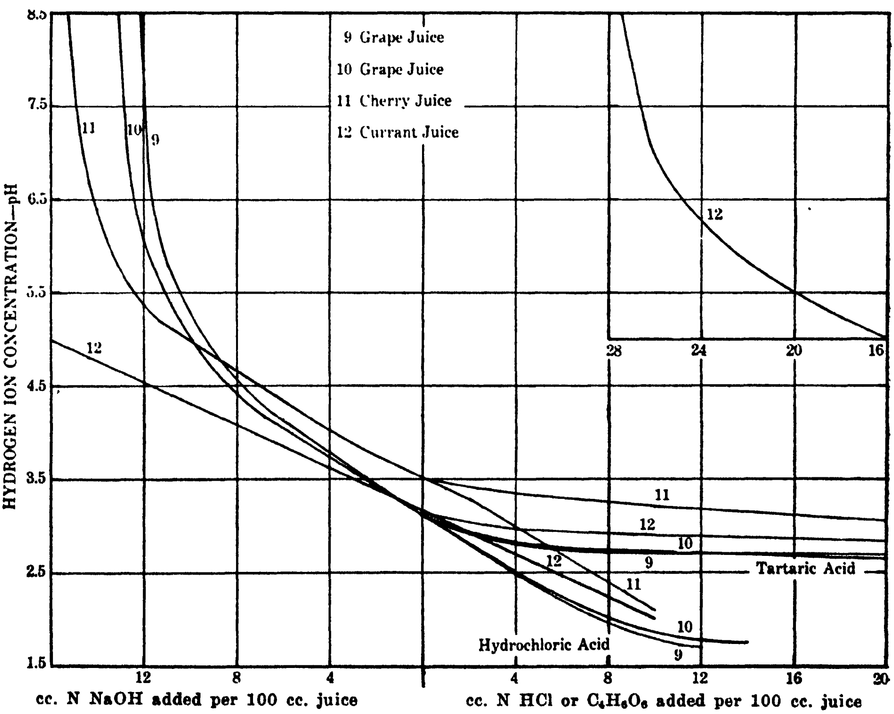

FIG. 3. Comparative buffering effect of grape, cherry, and red currant juices.

for apple juice. Some apple juices may have a high hydrogen-ion concentration, pH 3.1 to 3.2, but be so poorly buffered that they may have less effect than a juice of lower hydrogen-ion concentration, pH 3.3. There is little doubt but that elderberry juice would have its hydrogen-ion concentration raised by blending. The same would usually hold true in blending black raspberry and blackberry.

Bollinger (1942) titrated the acids of fruit juices electrometrically and suggested that juices be titrated to pH 8.1 in order that the end points would agree closely with the end points in phenolphthalein titration. The curves (Figs. 1, 2, and 3) show that there is some difference in acidity depending on the end point selected (Table 1). Since the steepest part of the curves is at about pH 8.1, one might attain a slightly greater variation in titratable acidity if either a lower or higher pH were selected as an end point.

SUMMARY

Samples of various fruit juices were titrated with sodium hydroxide, tartaric acid, and hydrochloric acid to establish buffer curves. Fruit juices vary considerably in their buffering effect. Therefore, one cannot predict the hydrogen-ion concentration of fruit-juice blends with any degree of accuracy. No correlation was noted between buffering effect and ash content.

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OBSERVATIONS ON THE BEHAVIOR OF STARCH GELS FROM DIFFERENT CLASSES AND VARIETIES OF WHEATS[1]

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(Received for publication, March 27, 1943)

In the past there has been a great deal of work published on the chemical and physical properties of starch. Much of this work is of historical interest but, in many instances, is very contradictory. Starch constitutes the greater portion of the wheat berry, while wheat flour contains even a higher percentage of starch.

Buchanan and Naudain (1923) thought that the superior bread-making qualities of hard wheat flours might be correlated in some manner with the high percentage of small starch grains found in hard wheat flours. Alsberg (1935) suggested that starch probably influences flour quality. Kozmin (1933) pointed out the importance of starch properties in connection with the quality of the crumb of bread. Woodruff and Webber (1933) considered a rigid paste of starch and water responsible to a large extent for the physical properties of baked products made with wheat flour.

Woodruff and MacMasters (1938) reported a detailed study of the physical properties of corn and wheat starches. They were unable to distinguish microscopically any varietal differences, any effect of the growing season, or any effect of starch treatment; however, wide differences in gel strength were evident.

Tanner and Englis (1940) reported further evidence of physical differences between hard and soft cornstarches. The hard starches formed more viscous pastes than soft starches of the same granular size. They found no appreciable difference in swelling power between starches except that one soft starch of large granular size did have higher swelling power. No correlation between relative viscosity and swelling power was evident.

The purpose of the investigation at this station was to determine the differences in gel strength of the starch from different classes and varieties of wheat, to study some of the factors that modify the gel strength, and to determine if any relationship exists between viscosity and gel strength and between the baking quality of the starches and gel strength.

Harris and Mason (1940) made a starch viscosity study, and the results of the baking quality of the starches were published by Harris and Sibbitt (1941).

[1] Contribution from the Departments of Animal and Human Nutrition and Cereal Technology, North Dakota Agricultural Experiment Station, Fargo, North Dakota. A progress report on Adams fund Project No. 24, "A Comparative Study of Certain Physical and Chemical Properties of Starch Prepared from Different Wheat Varieties." Published with the permission of the director of the station.

EXPERIMENTAL PROCEDURE

Three series of starches were prepared,[2] the first by a method essentially the same as that used by Woodruff and MacMasters (1938) and the other two by a procedure described by Harris and Sibbitt (1941).

The starches were gelatinized by a procedure essentially the same as the procedure outlined by Woodruff and MacMasters (1938). Woodruff and Nicoli (1931) found that starch must be heated to 90°C.(194°F.) or higher to obtain maximum gel strength. They thought that maximum gelatinization occurred in baking bread and in cooking potatoes and flour-thickened sauces.

Woodruff and Webber (1933) reported that 90°C. was the critical temperature of gel formation for five-per cent wheat-starch suspensions. They found that gels never formed below this temperature, that gels sometimes failed to form at 90°C. if the conditions of gelatinization were not ideal, and that the resulting gels were always weak and showed syneresis. They found a gradual improvement in gel form as the temperature increased from 90 to 95°C.(194 to 203°F.). Temperatures of 95°C., or above, gave the firmest gels with a five-per cent starch concentration.

Preliminary work in this laboratory indicated that six-per cent starch suspensions, which were gelatinized at 95°C., poured into 75 x 10 mm. petri dishes, and allowed to stand 10 hours before determining gel strength, were the most satisfactory. The starches were gelatinized in an electrically heated constant temperature bath equipped with a thermoregulator. Ethylene glycol (Prestone) was used in lieu of water as the heating fluid. A photograph and description of this apparatus were published by Harris and Knowles (1939). Because of the higher boiling point [197°C.(386.6°F.)] of Prestone, the starches could be rapidly gelatinized.[3] Woodruff and Webber (1933) reported the advisability of rapid gelatinization in order to obtain maximum gelling.

Trouble from condensed water dropping from the covers of the petri dishes onto the starch surface was overcome by covering the lower part of the petri dish with a filter paper,[4] and placing the cover of the petri dish over it. The filter paper absorbs the moisture which evaporates from the surface of the starch. The starch suspensions ready for gelatinization and gelatinized starch molded in the petri dishes are shown (Fig. 1).

The gel strengths were determined by a method fundamentally the same as the one developed by Binnington, Johannson, and Geddes (1939) for ascertaining the tenderness of macaroni. The apparatus developed by Bonney, Clifford, and Lepper (1931) for testing the tenderness of canned fruits and vegetables was modified by these workers.[5] A photograph of the instrument, as well as a detailed description of its construction, was published by Binnington *et al.* (1939).

[2] The starches were prepared by the Department of Cereal Technology, North Dakota Agricultural Experiment Station.

[3] The thermoregulator was set at 120°C.(248°F.), which is below the boiling point of Prestone.

[4] It is essential to use a rigid filter paper. C. S. & S. No. 598 was found to be satisfactory.

[5] This apparatus was constructed for this laboratory by D. S. Binnington.

FIG. 1. Apparatus used in preparing starch gels.

1. Starch suspension ready to be gelatinized.
2. Starch gels molded in petri dishes. (Note method of using filter paper.)

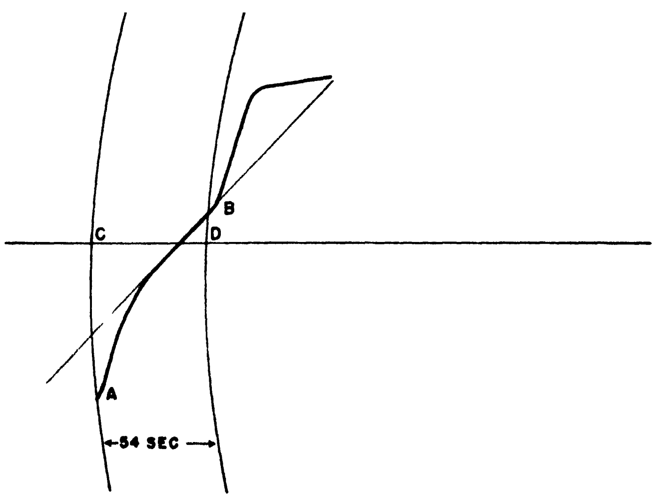

FIG. 2. A typical starch gel. *A* represents the starting point. *B* represents the point where the plunger breaks through the top surface of the starch gel. The time required to reach from *A* to *B* is represented by the line *CD*. This line represents the gel resistance in seconds.

A typical starch curve obtained on the kymograph is shown (Fig. 2). Binnington *et al.* (1939) described the special instruments used in evaluating these curves.

DISCUSSION OF RESULTS

In the first series of starches, made from wheats of the 1938 and 1939 crops, the amylodextrin fraction was removed from the starch. This fraction is considered by Sandstedt, Jolitz, and Blish (1939) to be largely the result of the action of beta-amylase on available starch.

Gel strengths and viscosities [6] of the experimental starches prepared from wheats grown in the 1938 and 1939 crop years are recorded (Tables 1 and 2). Gel strength of starches prepared from wheats grown in 1938 were low. Both Chiefkan and Mindum varieties ranked higher in 1939

TABLE 1

Average Gel Strength [1] and Viscosity [2] of Starches [3] Prepared From Different Varieties and Classes of Wheat (1938 Crop Year)

(Arranged in order of increasing gel strength)

Variety	Locality	Gel strength	Average of class	Viscosity at 90° C	Average of class
Soft red winter:		*sec.*	*sec.*	*cp.*	*cp.*
Trumbull	Indiana	55.3	55.3	1.40	1.40
Durum:					
Mindum	North Dakota	64.6	64.6	1.77	1.77
Hard red winter:					
Chiefkan	Kansas	73.3	......	1.35	......
Kanred	Kansas	75.4	74.35	1.37	1.36

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] Viscosities are recorded in centipoises. [3] The amylodextrin fraction was removed from the starch.

than in 1938. Trumbull, a soft red winter wheat, grown in 1938, had the lowest gel strength of the series. The results indicated that the starches prepared from soft wheats have lower gel strengths than those prepared from hard wheats. Tanner and Englis (1940) reported that hard starches appeared to form more viscous pastes than soft starches of approximately the same granule size. The series at this station included a greater number of hard red winter wheats than any other class. The range in gel strength within this class would appear to indicate a varietal difference. Woodruff and MacMasters (1938) reported that gel strength fluctuated widely with the variety of corn from which the starch was obtained. There was no correlation between the viscosity of the gelatinized starch and the gel strength.

Brimhall and Hixon (1939) reported a similar lack of correlation between rigidity and viscosity in their studies on cornstarch. Small differences in viscosities of cornstarches were observed by Woodruff and MacMasters (1938). The same investigators (1942) reported a definite lack of agreement between viscosity and gel strength, and they state that both gel strengths and viscosity tests are essential to measure accurately the physical properties of starch because these two tests measure two different

[6] The viscosity measurements were made by Walter Mason, Department of Cereal Technology, North Dakota Agricultural Experiment Station.

sets of properties in starch. Tanner and Englis (1940) found no correlation between swelling power and relative viscosity. Their results did, however, indicate the possibility that viscosity may decrease as swelling power increases.

Since there was no correlation between viscosity and gel strength, a second series of starehes was prepared with the amylodextrin fraction included in order to determine the relationship between gel strength and baking strength.[7] The starches were prepared by using a solution of .1 per cent sodium di-hydrogen phosphate (pH 6.8), as recommended by Dill and Alsberg (1924), rather than by the method of Woodruff and Mac-

TABLE 2

Average Gel Strength[1] and Viscosity[2] of Starches[3] Prepared From Different Varieties and Classes of Wheat (1939 Crop Year)

(Data arranged in order of increasing gel strength within classes)

Variety	Locality	Gel strength	Average of class	Viscosity at 90° C.	Average of class
Soft white:		*sec.*	*sec.*	*cp.*	*cp.*
Federation	Washington	69.60	69.60	1.83	1.83
Soft red winter:					
Wabash	Indiana	75.10	75.10	1.83	1.83
Hard red winter:					
Blackhull	Kansas	79.00	......	1.28	......
Cheyenne	Nebraska	79.40	......	1.34	......
Turkey	Kansas	85.60	......	1.24	......
Nebred	Nebraska	88.60	......	1.64	......
Chiefkan	Kansas	89.50	......	1.30	......
Tenmarq	Kansas	95.90	86.33	1.31	1.35
Emmer:					
Vernal Emmer	North Dakota	89.28	89.28	1.38	1.38
Hard white:					
Early Baart	Washington	90.50	90.50	1.21	1.21
Durum:					
Mindum	North Dakota	91.09	91.09	1.40	1.40

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] Viscosities are recorded in centipoises. [3] The amylodextrin fraction was removed from the starch.

Masters (1938), since the alcohol and ether used in the latter method are considered by some to change the character of the starch. This series included all the samples of the first series and additional varieties of hard red spring wheat. Experimental starches and a standard gluten were made into synthetic doughs, using the malt-phosphate-bromate, increased-sugar formula. The baking data on these starch-gluten doughs have been published by Harris and Sibbitt (1941) with a full description of the technique employed. This method is a modification of the one described by Sandstedt, Jolitz, and Blish (1939).

Gel strengths and baking strengths of the experimental starches in Series 2 (Tables 3 and 4) indicate that no relationship between baking and gel strengths is evident. The range in gel strength of varieties within the

[7] The baking tests were made by L. D. Sibbitt, Department of Cereal Technology, North Dakota Agricultural Experiment Station.

TABLE 3

Average Gel Strength[1] and Baking Strength of Starches[2] Prepared From Different Varieties and Classes of Wheat (1938 Crop Year)

(Data arranged in order of increasing gel strength)

Variety	Locality	Gel strength	Average of class	Loaf volume	Average of class
Soft red winter:		*sec.*	*sec.*	*c.c.*	*c.c.*
Trumbull	Indiana	40.8	40.8	178	178
Durum:					
Mindum	North Dakota	44.0	44.0	159	159
Hard red winter:					
Kanred	Kansas	59.7	......	186	......
Chiefkan	Kansas	73.2	66.45	180	183

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] The amylodextrin fraction was included in the starches.

TABLE 4

Average Gel Strength[1] and Baking Strength of Starches[2] Prepared From Different Varieties and Classes of Wheat (1939 Crop Year)

(Data arranged in order of increasing gel strength within classes)

Variety	Locality	Gel strength	Average of class	Loaf volume	Average of class
Soft red winter:		*sec.*	*sec.*	*c.c.*	*c.c.*
Wabash	Indiana	50.9	50.9	214	214
Soft white:					
Federation	Washington	54.1	54.1	158	158
Hard red spring:					
Mercury	North Dakota	46.6	......	173	......
Rival	North Dakota	57.0	......	173	......
Premier	North Dakota	64.3	......	170	......
Vesta	North Dakota	75.1	......	143	......
Thatcher	North Dakota	77.4	64.1	188	169.4
Hard white:					
Early Baart	Washington	65.4	65.4	151	151
Emmer:					
Vernal Emmer	North Dakota	77.3	77.3	179	179
Hard red winter:					
Tenmarq	Kansas	66.7	......	147	......
Blackhull	Kansas	74.1	......	160	......
Cheyenne	Nebraska	77.0	......	165	......
Turkey	Kansas	81.3	......	153	......
Nebred	Nebraska	81.3	......	174	......
Chiefkan	Kansas	86.0	77.7	155	159
Durum:					
Mindum	North Dakota	87.3	87.3	166	166

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] The amylodextrin fraction was included with the starch.

hard red winter and hard red spring classes is further evidence of varietal differences. Likewise, there is additional reason for postulating that soft wheat starches tend to have lower gel strengths than hard wheat starches.

The effect of including the amylodextrin fraction on gel strength (Table 5) shows a definite weakening of the starch gel regardless of variety

TABLE 5

Average Gel Strength[1] of Starches From Different Varieties and Classes of Wheat, Prepared With and Without Amylodextrin

Variety	Crop year	Gel strength of starch	Gel strength of starch + amylodextrin	Decrease in gel strength	Average decrease in gel strength
Soft red winter:		*sec.*	*sec.*	*sec.*	*sec.*
Trumbull	1938	55.3	40.8	14.5	14.5
Durum:					
Mindum	1938	64.6	44.0	20.6	20.6
Hard red winter:					
Chiefkan	1938	73.3	73.2	0.1	......
Kanred	1938	75.4	59.7	15.7	7.9
Soft white:					
Federation	1939	69.6	54.1	12.5	12.5
Soft red winter:					
Wabash	1939	75.1	50.9	24.2	24.2
Hard red winter:					
Blackhull	1939	79.0	74.1	4.9	
Cheyenne	1939	79.4	77.0	2.4	......
Turkey	1939	85.6	81.3	4.3	......
Nebred	1939	88.6	81.3	7.3	......
Chiefkan	1939	89.5	86.0	3.5	......
Tenmarq	1939	95.9	66.7	29.2	8.6
Emmer:					
Vernal Emmer	1939	89.28	77.3	11.98	11.98
Hard white:					
Early Baart	1939	90.50	65.4	25.10	25.10
Durum:					
Mindum	1939	91.09	87.3	3.79	3.79

[1]Gel strength is recorded in the number of seconds required to break the surface of the starch gel.

TABLE 6

Average Gel Strength of Wheat Starches With Added Amylodextrin

Variety	Locality	Gel strength				
		Starch + natural amylodextrin	Pure starch	Starch + 5 per cent amylodextrin	Starch + 10 per cent amylodextrin	Starch + 15 per cent amylodextrin
		sec.	*sec.*	*sec.*	*sec.*	*sec.*
Thatcher	North Dakota	24.7	55.0	51.0	49.3	30.0
Rival	North Dakota	42.7	57.3	34.0	20.3	15.5
Vesta	North Dakota	53.3	66.0	57.7	46.5	43.5

or class. The effect of including the amylodextrin fraction varies widely from class to class. Within the hard red winter varieties for the 1939 crop year, the effect of including the amylodextrin fraction is fairly consistent, with the exception of Tenmarq. These starches, with the exception of Ten-

marq, were not weakened greatly when the amylodextrin fraction was included. This might be attributed to a low content of beta-amylase. Unfortunately, this study did not include enough varieties of other classes of wheat to make any statement concerning them.

Graduated amounts (5, 10, and 15 per cent) of amylodextrin were added to wheat starch from which the amylodextrin fraction had been removed. Starch from three varieties (Rival, Vesta, and Thatcher) were treated in this manner, and the gel strengths determined. The results (Table 6) show clearly the weakening effect of amylodextrin upon starch.

TABLE 7

Average Gel Strength[1] and Baking Strength of Starches[2] Prepared From Different Varieties and Classes of Wheat (1940 Crop Year)

(Data arranged in order of increasing gel strength within classes)

Variety	Locality	Gel strength	Average of class	Loaf volume	Average of class
Soft red winter:		*sec.*	*sec.*	*c.c.*	*c.c.*
Mitchikoff	....	35.83	....	148	....
Wabash	....	39.50	....	139	....
American Banner	....	40.56	38.63	142	143.00
Hard red spring:[3]					
Renown (new)	North Dakota	42.86	....	134	....
Regent	North Dakota	44.39	....	131	....
Ceres	North Dakota	46.35	....	138	....
Premier	North Dakota	47.95	....	128	....
Nordhaugen	North Dakota	48.04	....	130	....
Brandon 123	North Dakota	48.28	....	125	....
Marquis	North Dakota	48.83	....	133	....
Pilot 13	North Dakota	51.49	....	132	....
Merit	North Dakota	51.91	....	127	....
Pilot B	North Dakota	53.64	....	132	....
Rival	North Dakota	54.03	....	128	....
Thatcher	North Dakota	55.62	....	136	....
Vesta	North Dakota	57.34	50.06	126	130.76
Hard red winter:					
Tenmarq	....	54.11	....	134	....
Chiefkan	....	54.25	....	137	....
Blackhull	....	58.17	....	137	....
Nebred	....	60.50	....	155	....
Turkey	....	60.75	57.56	149	142.40

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] The amylodextrin fraction was included with the starch. [3] The wheats were grown at Dickinson, Mandan, Langdon, and Fargo, with the exception of Brandon 123 and Regent, which were grown at Fargo and Langdon, only.

There is apparently a varietal difference in the extent of the weakening effect of amylodextrin. These results substantiate the results shown in Table 5, which had indicated the above facts.

The third series of experimental starches included the amylodextrin fraction.[8] This series included soft red winter, hard red winter, and hard red spring wheats grown in 1940. The gel strengths and baking strengths of the experimental starches in Series 3 (Table 7) indicate that there was no apparent relationship between baking strength and gel strength. Since

[8] This series of starches was prepared by the method of Dill and Alsberg (1924).

this is in agreement with the results of the previous year, it seems reasonable to assume that no relationship exists between baking strength and gel strength.

Eleven varieties of the hard red spring wheats were grown under comparable conditions in four different regions of the state (1940 crop year); their gel strengths are shown (Table 8). As a rule, the starches of the wheats grown at Fargo had higher gel strengths than starches from similar

TABLE 8

Average Gel Strength[1] of Starches[2] From 11 Varieties of Hard Red Spring Wheat Grown in Four Different Localities

(Arranged in order of increasing gel strength)

Variety	Gel strength of starch in seconds				
	Fargo	Dickinson	Mandan	Langdon	Average for variety
Renown (new)	46.75	46.12	41.38	37.17	42.86
Ceres	49.88	49.00	48.17	38.33	46.35
Premier	52.60	51.36	49.00	45.22	47.95
Nordhaugen	54.40	49.75	45.00	43.00	48.04
Marquis	53.28	47.00	54.20	40.80	48.83
Pilot 13	55.62	59.60	53.25	37.50	51.49
Merit	51.28	52.40	54.25	49.70	51.91
Pilot B	58.50	51.50	55.67	48.89	53.64
Rival	71.50	56.33	47.60	40.70	54.03
Thatcher	72.00	56.12	52.50	41.88	55.62
Vesta	67.67	56.38	61.67	43.62	57.34
Average for station	57.59	52.32	51.15	42.44	....

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] The amylodextrin fraction was included with the starch.

TABLE 9

Average Gel Strength[1] of Starches[2] Prepared From Thatcher Wheat Harvested at Different Stages of Maturity (1941 Crop Year)

Variety	Locality	Harvest date	Gel strength	Moisture
			sec.	*pct.*
Thatcher	Fargo	7/19/41	27.3	12.8
Thatcher	Fargo	7/24/41	39.0	11.0
Thatcher	Fargo	7/28/41	53.3	11.7
Thatcher	Fargo	9/ 3/41	56.3	10.8

[1] Gel strength is recorded in the number of seconds required to break the surface of the starch gel. [2] The amylodextrin fraction was included with the starch.

wheats grown in other localities. The Langdon wheats gave consistently lower gel strengths. Woodruff and MacMasters (1938) reported that gel strengths of cornstarches fluctuated with the conditions under which the corn was grown. Further evidence of the effect of environmental and climatic conditions is shown by comparing the average gel strengths of the varieties for 1939 and 1940. The wheats yielded stronger starches in 1939.

Further tests were made in the 1941 crop year to ascertain the effect of the date of harvest on the strength of the wheat starch gel. Results of

a few tests (Table 9) made on one hard red spring wheat variety, Thatcher, indicate an increase in gel strength with increasing maturity. The strength of the gel increased at a rapid rate between July 19 and 28. The rapid increase in gel strength leveled off approximately on July 28. There was only a small difference between the starch of wheat harvested July 28 and the starch of wheat harvested August 3.

SUMMARY AND CONCLUSIONS

These results indicate that there is a class difference in wheat-starch gels. Starches of hard wheats have stronger gels than starches of soft wheats.

There is some evidence of varietal differences in wheat-starch gel strength.

There is no apparent relationship between viscosity and starch gel strength.

The inclusion of the amylodextrin fraction apparently weakens the starch gel, but not to the same extent in each class of wheat nor in each variety within a class.

These data do not indicate any relationship between baking strength and gel strength.

There is evidence that environmental conditions during growth may have an effect on the strength of the starch gel and stage of maturity may influence gel strength.

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THIAMIN CONTENT OF FRESH AND FROZEN PEAS AND CORN BEFORE AND AFTER COOKING[1,2]

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Although some reports have been found in the literature on thiamin content of peas and corn, little concerning the fate of this vitamin during cooking can be found.

The purpose of this study was to determine the effect of the several cooking methods on the thiamin content of fresh and quick-frozen peas and corn from the same lots.

REVIEW OF LITERATURE

Peas: The values in the literature indicate that fresh peas may contain from approximately two micrograms to over eight micrograms of thiamin per gram. Rose and Phipard (1937) found that peas were a rich source of thiamin, containing approximately three Sherman-Chase units per gram. These workers reported that there was no loss owing to freezing and a 26-per cent loss owing to 15 minutes' cooking.

Baker and Wright (1938) determined thiamin by the Bradycardia method and reported that green peas contained from 4.8 to 8.4 micrograms per gram when raw and 2.4 to 3.6 micrograms per gram when cooked. Booher and Hartzler (1939) found that green peas contained 3.9 micrograms of thiamin per gram.

Fincke (1939) blanched peas for different lengths of time at different temperatures prior to freezing and found that the general trend was toward a lower thiamin content of peas scalded for longer periods of time at higher temperatures. She observed no loss owing to holding peas after vining, prior to freezing, from four to eight hours at room temperature. Fincke also reported that peas grown under the same soil and climatic conditions and frozen by the same methods varied in thiamin content.

Fellers, Esselen, and Fitzgerald (1940) determined the thiamin content of Laxton peas by the rat-growth method. When fresh, the peas contained 3.99 micrograms of thiamin per gram; when frozen after blanching for 50 seconds at 100°C.(212°F.), they contained 3.9 micrograms per gram. Canned peas from the same lot, blanched 50 seconds at 100°C. then processed for five minutes at 115.5°C.(240°F.), contained 1.35 micro-

[1] This paper is based on part of the research work conducted by Barbara Barnes in partial fulfillment of the requirements for a master's degree. The work was supported in part by a fellowship offered by the Frosted Foods Sales Corporation, New York City.

[2] Approved by the director of the New York State Agricultural Experiment Station as Journal Paper No. 543, February, 1943.

grams of thiamin per gram. The liquid from these cans contained 1.77 micrograms per gram.

Richardson, Mayfield, and Davis (1940) found that frozen peas of the Laxton variety contained 1.02 micrograms of thiamin per gram; peas, frozen and boiled for 30 minutes, contained .85 microgram per gram; home-canned peas, stored six months and then reheated in their own juice for 10 minutes, contained .82 microgram per gram.

Aughey and Daniel (1940) reported that frozen and stored Alderman peas, blanched by scalding for one minute at 88 to 93°C.(190.4 to 199.4°F.), contained 6.7 micrograms of thiamin per gram. They also found that raw Laxton peas contained 4.5 micrograms per gram, and Laxton peas simmered for 12 minutes, with or without soda, contained 3.6 micrograms per gram. Pyke (1940) determined the thiamin content of fresh peas by the thiochrome method and found 1.2 I. U. (3.6 micrograms) per gram.

Moyer and Tressler (1942) used Thomas Laxton peas to compare methods of thiamin analysis. The thiochrome method indicated a thiamin content of 2.6 micrograms per gram; the biological assay, 4.22 and 3.06 micrograms per gram; and fermentation after sulfite cleavage, 2.69 micrograms of thiamin per gram.

Moyer and Tressler (1943) have also reported a study of the thiamin content of Thomas Laxton peas at various stages in processing prior to and after freezing. They found that peas freshly harvested contained 4.11 micrograms per gram; before blanching (there was a delay between harvesting and blanching), 3.08 micrograms; after blanching, 2.92 micrograms; after quality separation, 2.87 micrograms; before packaging, 2.84 micrograms; and after freezing, 2.83 micrograms of thiamin per gram. They repeated this same type of study with Telephone peas and found that freshly harvested peas contained 4.33 micrograms of thiamin per gram; before blanching, 4.21 micrograms; after blanching, 3.61 micrograms; after quality separation, 3.42 micrograms; before packaging, 2.95 micrograms; and after freezing, 3.36 micrograms of thiamin per gram.

Corn: Richardson, Mayfield, and Davis (1937) reported the effect of home preservation on the thiamin content of Golden Bantam sweet corn. They found that raw corn contained 1.98 micrograms per gram; when dried, soaked, and cooked 30 minutes it contained .54 microgram; preserved by salting, soaked, and cooked it contained .54 microgram; preserved by fermentation in brine, soaked, and cooked it contained .27 microgram; and when canned in a pressure cooker for 75 minutes at 121.1°C.(250°F.) it contained .54 microgram of thiamin per gram.

Pyke (1939) found that sweet corn on the cob contained 1.44 micrograms of thiamin per gram. Booher and Hartzler (1939) reported that Country Gentleman corn contained 1.20 micrograms of thiamin per gram.

EXPERIMENTAL PROCEDURE

Vegetables Studied: Peas of the Thomas Laxton, Gradus, and Alderman varieties and two lots of corn of the Golden Cross variety were used in this study. The corn was obtained on two different days and will be referred to in this report as Lot 1 and Lot 2. The peas were harvested during the month of June, 1941, and the corn during the month of August

near Mt. Morris, New York. They were a part of the regular commercial pack prepared and frozen for the Frosted Foods Sales Corporation. Peas were harvested, vined, water-blanched for 60 seconds, water-cooled, quality separated, and packaged. Corn was harvested, husked, steam-blanched, water-cooled, graded, cut from the cob, and packaged. These vegetables were frozen in a Birdseye Multiplate Freezer. The approximate temperature of the plates of this machine was —33.3°C.(—28°F.). The vegetables were stored for several days in a commercially refrigerated warehouse, maintained at —17.8°C.(0°F.), and then were stored in a cold room which was maintained at —21.7 to —23.3°C.(—7 to —10°F.).

Sampling: Preliminary work to determine the best methods of sampling was conducted as follows: (1) peas from the Experiment Station gardens were analyzed (a) immediately after harvesting, (b) after holding unshelled for two hours, (c) after holding unshelled for five hours, and (d) after holding shelled for five hours at room temperature; (2) peas grown in the same field near Mt. Morris were taken (a) directly from the load before shelling and (b) from the boxes after being vined and partially cleaned, iced, and transported approximately 55 miles to Geneva for analysis; and (3) corn from the Experiment Station gardens was analyzed (a) immediately after harvesting and (b) after standing unhusked for five hours at room temperature.

Neither vegetable lost thiamin under any of the above conditions. Therefore, samples were taken from the boxes of vined, partially cleaned, iced peas, after transporting them to Geneva (not more than four hours were required), and used for raw samples and for cooking studies. From 15 to 20 ears of corn were taken from the load at Mt. Morris, iced and transported to Geneva, where corn for raw samples and cooking studies was cut from the cob. Samples for cooking were also taken after the corn had been blanched and cut from the cob at the freezing plant. This was thought advisable since the raw corn cut from the cob, without previous blanching, was very milky and not comparable with the frozen material.

To insure having the fresh and frozen vegetables used in this study from the same lot, the vegetables were followed through the freezing plant and packages were labeled to be used later for cooking studies.

Chemical Determination: The modified thiochrome method of Moyer and Tressler (1942) for determining thiamin in vegetables was used. This is an adaptation of the procedure suggested by the Research Corporation Committee in conjunction with the Committee on Vitamin Fortification of the American Association of Cereal Chemists (1943). The thiamin content of the raw, frozen, and cooked vegetables and of the cooking water was determined.

Preparation for Cooking: The frozen peas and corn were started to cook while they were still solidly frozen. The packages of both were pounded on the table to separate the peas and corn sufficiently so that samples for analysis could be removed easily.

Cooking Studies: In all the cooking studies a one-quart enamel pan, six inches in diameter and three inches deep, with a pyrex cover was used. The pan and a gas burner were placed on a balance so that the weight of the pan and its contents could be obtained during each stage

of cooking. A manometer was connected between the gas supply and the burner so that it was possible to duplicate the amount of evaporation in the various cookings—Fenton, Tressler, and King (1937).

Three hundred grams of vegetable (approximately four servings) were cooked in each case. The vegetables were dropped into rapidly boiling water, and the cover was placed on the pan. The flame was adjusted so that it took five minutes for the contents of the pan to return to the boiling point. The peas were boiled gently for four minutes and the corn for two minutes. Fresh peas came to the boil in two and one-half minutes and were boiled for eight minutes; fresh corn came to the boil in two minutes and was boiled for four minutes. Blanched corn came to the boil in two minutes and was boiled for two minutes.

Fresh peas used in this study required a shorter period of boiling than is usually recommended to insure a "done" product. However, none of the peas were mature enough to require the longer cooking time. The fact that these peas were very tender may also explain why the judges preferred the frozen peas boiled for four minutes rather than those boiled for five to seven minutes, as directed on the package.

The corn, also, was not as mature as that frequently used. The judges selected the corn cooked for the lengths of time used in this study as the most satisfactory product.

The cooked vegetables were drained for 30 seconds in an enamel colander, the cooked vegetables weighed, and the weight of the cooking water determined.

Fresh and frozen peas were boiled in 100 grams of water; frozen peas of the Gradus variety were also boiled in 50 and 600 grams of water. Fresh, raw corn, cut from the cob; blanched, unfrozen corn cut from the cob; and blanched, frozen corn were boiled in 100 grams of water. One lot of corn was also boiled in 600 grams of water.

DISCUSSION AND RESULTS

Effect of Holding on Thiamin Content of Fresh Peas and Corn: No loss of thiamin from fresh peas and corn was observed when the products were allowed to stand as long as five hours at room temperature. This result indicated that peas for raw and cooking samples could be held, without introducing an error, for a few hours before the laboratory work was done and made it possible (1) to obtain a representative sample of the peas, which had been vined and were ready to be frozen, by taking them from the boxes at the freezing plant and (2) to follow the peas through the plant and obtain frozen samples from the same lot. Fresh, vined peas were held on ice to keep them in the best possible condition and they were taken to Geneva where cooking studies and analyses were done. Unhusked ears of corn were taken from the loads at Mt. Morris and were also iced during the transportation to Geneva.

Effect of Freezing and Storage on Thiamin Content of Peas and Corn: The amounts of thiamin found in fresh, frozen, and stored peas and corn are shown (Table 1). During the processing before freezing some thiamin was lost, probably by solution into the blanching and cooling water.

Both frozen peas and corn were stored for one year at —21.7 to —23.3°C. (—7 to —10°F.). No appreciable loss of thiamin was observed for this period of time (Table 1).

Effect of Cooking Upon Thiamin Content of Three Varieties of Fresh and Frozen Peas: Regardless of the variety, little difference was noted in the per cent solution, retention, or loss of thiamin when 300 grams of fresh peas were boiled in 100 grams of water. Peas of the Alderman variety retained 83 per cent, of the Gradus variety 84 per cent, and of the Laxton variety 80 per cent of the thiamin present in the raw samples (Table 2). No destruction of the thiamin was observed. The apparent increase in thiamin content (two per cent) of the cooked peas plus the cooking water is within experimental error.

TABLE 1

Thiamin Content of Three Varieties of Fresh, Frozen, and Stored-Frozen Peas and One Variety of Sweet Corn

Vegetable	Variety	Thiamin content		
		Fresh vegetable	Frozen vegetable	
			No storage	One-year storage
		μg./gm.	*μg./gm.*	*μg./gm.*
Peas	Gradus	4.40	3.36	3.61
	Laxton	4.50	3.74	3.70
	Alderman	3.95	2.79	2.80
Corn	Golden Cross			
	Lot 1	1.55	1.28	1.24
	Lot 2	1.75	1.26	1.22

During the cooking of the frozen peas by the above method there was no destruction but a solution to the cooking water of approximately one-fourth of the thiamin (Table 2). These values for thiamin retention in the frozen peas are somewhat lower than those found for fresh peas. A comparison of the amounts of water left at the end of the cooking period shows that there was more water remaining after the frozen peas were cooked and larger quantities of thiamin were found in solution. As will be shown later in this study, a smaller amount of water caused a greater retention of thiamin in the vegetable. An increased solution of thiamin when larger amounts of water were used was also found by Fenton, Barnes, Moyer, Wheeler, and Tressler (1942), who cooked dehydrated vegetables in varying amounts of water.

Effect of Increasing Cooking Water Upon Thiamin Content of Frozen Peas: Since all varieties of peas showed the same trend in previous cooking studies, only the Gradus variety was cooked in different amounts of water. The results of this study (Table 2) showed an increased per cent solution of thiamin owing to larger volumes of water used in cooking up to the point where the peas were completely immersed in water. After this stage was reached the increase in the amount of water had little effect on the solution of thiamin from peas. This indicated that the use of the smallest amount

TABLE 2

Summary of Effect of Cooking[1] on Thiamin Content of Fresh and Frozen Peas and Corn

Vegetable	Variety	Weight of water	Weight of cooked vegetable	Weight of cooking water [2]	Boiling time	Thiamin content				
						Uncooked vegetable	Cooked vegetable	Cooking water	Retention	Solution
		gm.	*gm.*	*gm.*	*min.*	*µg./gm.*	*µg./gm.*	*µg./gm.*	*pct.*	*pct.*
Fresh peas	Alderman	100	279	94	8	3.95	3.50	2.13	83	17
	Gradus	100	264	95	8	4.40	4.20	2.56	84	18
	Laxton	100	262	98	8	4.50	4.10	2.81	80	20
Frozen peas	Alderman	100	272	120	4	2.80	2.32	1.50	75	22
	Gradus	50	256	142	4	3.61	3.47	2.55	82	21
	Gradus	100	247	91	4	3.61	3.16	2.11	72	28
	Gradus	600	253	634	4	3.61	2.47	0.57	64	34
	Laxton	100	269	125	4	3.70	3.29	2.02	79	22
Raw corn, Lot 1	Golden Cross	100	375	......	10	1.55	0.95	......[3]	78	....
Raw corn, Lot 2	Golden Cross	100	350	......	10	1.75	1.18	......[3]	80	....
Blanched, unfrozen, Lot 1	Golden Cross	100	300	64	4	1.30	1.10	0.89	85	15
Blanched, unfrozen, Lot 2	Golden Cross	100	310	60	4	1.25	0.98	1.20	81	15
Blanched, frozen, Lot 1	Golden Cross	100	299	94	2	1.24	0.94	0.64	76	25
Blanched, frozen, Lot 2	Golden Cross	100	314	79	2	1.22	0.92	1.00	78	22
Blanched, frozen, Lot 2	Golden Cross	600	305	583	2	1.22	0.76	0.21	63	34

ree hundred grams of vegetable were cooked in each study. [2] At the end of the cooking period. [3] Too little water was left to analyze.

of water possible in cooking resulted in the greatest retention of thiamin in the cooked vegetable.

Little difference could be detected in the flavor of the cooked peas whether 50 or 100 grams of water were used. When 600 grams of water were used the peas had a much weaker, washed-out flavor. The green color was well retained in all cases.

The cooking water remaining after the peas had been boiled in 50 grams of water was a good source of thiamin, containing 2.55 micrograms per gram. When 600 grams of water were used it contained only .57 microgram of thiamin per gram, even though 13 per cent more thiamin had gone into solution.

Effect of Cooking on Thiamin Content of Fresh, Blanched, Unfrozen and Blanched, Frozen Sweet Corn: The effect of cooking on blanched, unfrozen and blanched, frozen corn was compared since raw corn, cut from the cob, is not comparable to frozen corn which is cut from the cob after blanching.

When fresh corn was cooked, approximately 80 per cent of the thiamin remained in the corn. Too small an amount of water remained to analyze.

Very little difference in the retention and solution of thiamin occurred when blanched, unfrozen and blanched, frozen corn were cooked (Table 2). The larger volume of water remaining after cooking frozen corn would account for the slightly higher per cent solution and the lowered thiamin content.

Effect of Increasing Cooking Water on Thiamin Content of Frozen Corn: Corn from Lot 2 was cooked in 100 and in 600 grams of water. It was not possible to use less cooking water and prevent scorching at the end of the cooking period. With the increase in the amount of water the retention of thiamin in the corn decreased 15 per cent (Table 2). The remainder of the thiamin was found in the cooking water.

SUMMARY

Fresh peas did not lose thiamin when they were held for five hours, shelled or unshelled, at room temperature. Fresh, unhusked corn showed no loss of thiamin during a five-hour storage period at room temperature.

Cooked, frozen peas were a fair source of thiamin.

Storage of frozen peas and corn at —17.8 to —23.3°C.(0 to —10°F.) for one year did not result in a loss of thiamin.

There was no loss of thiamin during the cooking of fresh and frozen peas and corn. Different cooking methods resulted in a 64 to 84 per cent retention of thiamin in the peas and a 63 to 85 per cent retention in the corn. Variety was not a factor influencing the amount of solution or retention of thiamin in the cooking of fresh or frozen peas. Two lots of corn, harvested on different days and cooked by the same method, gave approximately the same per cent retention of thiamin.

Increasing the amounts of water used in cooking peas and corn caused an increase in the amount of thiamin dissolved in the cooking water. The smaller volumes of water used for cooking peas and corn were found to be more concentrated sources of thiamin than the larger volumes.

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A COMPARATIVE STUDY OF ONE-PER CENT AND FIVE-PER CENT SOLUTIONS OF 30- TO 40-MESH GELATINS FOR BACTERIOLOGICAL EXAMINATION

TECHNICAL COMMITTEE OF THE EDIBLE GELATIN MANUFACTURERS' RESEARCH SOCIETY OF AMERICA[1 2]

(Received for publication, March 27, 1943)

Various methods of examining gelatin bacteriologically have been in use in the United States and Canada. These methods have varied in the amount of sample used in preparing the original solution, the method of preparing the solution itself, the culture media employed, the time and temperature of incubation, the method of counting, and various other items. As gelatin is often purchased in accord with certain bacteriological specifications, it is a matter of considerable importance to have recognized standard procedures of examination in order that the results obtained in different laboratories may be comparable. The attention of gelatin manufacturers was focused on this problem not only because of the variations in procedures and requirements but also because the Committee on Standard Methods for the Bacteriological Examinátion of Dairy Products and Frozen Desserts of the American Public Health Association proposed to include a standard procedure for edible gelatin in the next issue of *Standard Methods.* Since several members of the Technical Committee of the Edible Gelatin Manufacturers' Research Society of America are also members of the A.P.H.A. Committee on Standard Methods of Examining Gelatin, it was thought desirable to initiate some comparative studies in the laboratories of the component companies of the Edible Gelatin Manufacturers' Research Society. As the amount of the original sample used for analysis was a matter of divergent opinion, it was thought best to concentrate on this item and to keep all other factors constant. Only one of the laboratories used 10-gram samples for analysis as a routine procedure. All were agreed, however, that such quantities definitely presented analytical difficulties and should therefore be ruled out. It was decided to compare one-per cent *versus* five-per cent solutions of gelatin since some of the laboratories used the lower concentration and others favored the higher.

Three specially prepared high-count gelatins and three low-count gelatins were employed in this comparative study. Each gelatin was finely ground and was capable of passing through 30- to 40-mesh screens. Each batch of gelatin was thoroughly mixed during manufacture. A five-pound sample of each gelatin was obtained using aseptic precautions and, after

[1] The companies making up the Edible Gelatin Manufacturers' Research Society of America are as follows: American Agricultural Chemical Company; Atlantic Gelatin Company; Kind and Knox Gelatine Company; Milligan and Higgins Corporation; UCOPCO Gelatine (United Chemical and Organic Products Company); Eastman Gelatine Corporation.

[2] This paper was prepared by Dr. Murray P. Horwood, Consulting Bacteriologist, for the Atlantic Gelatin Company, Woburn, Mass., who acted as referee.

further mixing in the laboratory, was divided into five equal portions and delivered to the co-operating laboratories in new, clean, smooth, sealed cardboard containers provided with metal caps. Every effort was made to prepare samples that were uniform. The samples were further mixed in each co-operating laboratory, after which definite amounts were removed for analysis. The procedure employed for analysis was identical in each case.

EXPERIMENTAL PROCEDURE

The one-gram and five-gram portions of gelatin were weighed aseptically in sterile, aluminum boats on a sensitive balance. The one-gram samples were introduced into bottles containing 99 ml. of sterile, distilled water, while the five-gram portions were introduced into bottles containing 95 ml. of sterile, distilled water. The gelatin was thoroughly wetted at room temperature in each case and introduced promptly into a water bath maintained at 45°C.(113°F.), mixed frequently, and brought into solution as quickly as possible. After that, duplicate one-ml. amounts were removed and introduced into separate, sterile Petri dishes. In the meantime, standard nutrient agar prepared from the dehydrated product manufactured by the Digestive Ferments Company in accord with the requirements of the A.P.H.A. Committee on Standard Methods for the Examination of Water, was brought into solution and cooled to 45°C. Approximately 10 to 12 ml. of the melted nutrient agar were added to each Petri dish containing one-ml. amounts of the one-per cent and five-per cent solutions of the gelatins and their diluents. A 1:10 dilution of the one-per cent solutions of gelatin was prepared using 11 ml. of the original solution in 99 ml. of sterile, distilled water. The 1:100 dilutions of the five-per cent solutions were prepared by using one ml. of the original solution in 99 ml. of sterile, distilled water. The plates were inverted and incubated at 37°C. (98.6°F.) for 48 hours and counted with the aid of a Quebec Colony Counter. Altogether six samples of gelatin were studied. Each sample was examined by preparing five separate one-per cent solutions and five separate five-per cent solutions. Duplicate one-ml. portions were examined for total count from each original solution and its dilution. For the sake of brevity the individual results in duplicate for the five one-per cent and five five-per cent cent solutions of each sample of gelatin and their dilutions are omitted here. Instead there are recorded only the minimum, maximum, and mean counts for each series and for each dilution (Table 1).

DISCUSSION

The results obtained indicate quite clearly that the counts vary appreciably. Sometimes the counts obtained on the one-per cent solutions were materially higher than those obtained on the five-per cent solutions; and sometimes the reverse was true. In general, however, the range of the count per gram obtained with the one-per cent samples is in the same category as the range obtained with the five-per cent samples. Not only is this conclusion supported by the results obtained in a given laboratory, but it is supported by the results obtained in the various laboratories on the same samples. While identical results were not obtained, they were within the same numerical zone. This is what one should expect in view of the

TABLE 1

Comparative Bacteriological Findings[1] in Bacteria per Gram of 30- to 40-Mesh Gelatin on 1.5 Per Cent Nutrient Agar at 37°C.(98.6°F.) After 48 Hours

	American Agricultural Chemical Company	Atlantic Gelatin Company	Kind and Knox Gelatine Company	Milligan and Higgins Corporation	Ucopco Gelatine
Sample A—One-Gram Portions					
Dilution 0					
Minimum	5,100	9,300	Too numerous	8,700	7,300
Maximum	9,000	37,000	Too numerous	13,500	13,500
Mean	6,740	16,260	Too numerous	10,360	10,490
Dilution 1:10					
Minimum	3,850	11,500	15,000	9,000	8,500
Maximum	12,100	41,000	30,500	14,500	13,500
Mean	7,150	18,400	27,900	12,040	11,300
Sample A—Five-Gram Portions					
Dilution 0					
Minimum	2,960	10,000	30,000	Too numerous	4,600
Maximum	6,120	16,100	48,000	Too numerous	8,250
Mean	4,464	12,480	39,125	Too numerous	4,900
Dilution 1:100					
Minimum	2,000	14,000	25,000	14,000	6,000
Maximum	10,000	26,000	35,000	18,000	9,000
Mean	7,800	21,600	30,000	16,000	7,000
Sample B—One-Gram Portions					
Dilution 0					
Minimum	38,700	32,000	....	38,000	30,000
Maximum	89,200	46,600	....	44,000	80,000
Mean	58,560	39,520	....	41,700	53,400
Dilution 1:10					
Minimum	55,000	43,000	....	44,000	38,000
Maximum	64,900	59,500	....	56,000	135,000
Mean	59,510	51,700	....	51,250	82,100
Sample B—Five-Gram Portions					
Dilution 0					
Minimum	81,800	22,600	....	Too numerous	22,000
Maximum	112,500	34,500	....	Too numerous	32,000
Mean	102,820	29,100	....	Too numerous	24,640
Dilution 1:100					
Minimum	60,000	48,000	....	50,000	40,000
Maximum	100,000	74,000	....	58,000	181,000
Mean	74,000	58,000	....	55,200	95,800
Sample C—One-Gram Portions					
Dilution 0					
Minimum	9,850	14,600	1,800	15,000	9,100
Maximum	14,600	22,200	3,200	21,000	10,750
Mean	12,920	18,120	2,470	17,600	10,000
Dilution 1:10					
Minimum	11,650	12,000	2,500	16,000	9,000
Maximum	17,600	34,500	4,000	22,000	22,000
Mean	13,280	24,200	3,000	19,500	16,000
Sample C—Five-Gram Portions					
Dilution 0					
Minimum	8,960	41,700	2,350	Too numerous	7,200
Maximum	9,930	89,800	4,400	Too numerous	12,700
Mean	9,313	57,100	3,100	Too numerous	10,530
Dilution 1:100					
Minimum	10,000	98,000	2,000	23,500	19,000
Maximum	25,500	138,000	9,000	30,000	22,500
Mean	18,900	118,400	4,000	26,000	20,700

[1] Average of five analyses.

TABLE 1 *(Concluded)*

Comparative Bacteriological Findings[1] in Bacteria per Gram of 30- to 40-Mesh Gelatin on 1.5 Per Cent Nutrient Agar at 37°C.(98.6°F.) After 48 Hours

	American Agricultural Chemical Company	Atlantic Gelatin Company	Kind and Knox Gelatine Company	Milligan and Higgins Corporation	Ucopco Gelatine
Sample E—One-Gram Portions					
Dilution 0					
Minimum	800	450	600	350	650
Maximum	1,400	1,150	1,100	525	1,200
Mean	1,090	825	820	430	920
Dilution 1:10					
Minimum	550	Too few	0	Too few	[illegible]
Maximum	2,200	Too few	5,000	Too few	4,000
Mean	1,320	Too few	1,900	Too few	2,000
Sample E—Five-Gram Portions					
Dilution 0					
Minimum	580	480	300	320	200
Maximum	690	640	580	440	600
Mean	650	552	416	388	332
Dilution 1:100					
Minimum	0	Too few	200	Too few	1,000
Maximum	4,000	Too few	600	Too few	3,500
Mean	1,600	Too few	280	Too few	2,100
Sample F—One-Gram Portions					
Dilution 0					
Minimum	0	0	0	0	0
Maximum	100	100	200	50	400
Mean	70	30	100	20	130
Dilution 1:10					
Minimum	0	Too few	0	Too few	0
Maximum	1,000	Too few	0	Too few	1,000
Mean	400	Too few	0	Too few	300
Sample F—Five-Gram Portions					
Dilution 0					
Minimum	0	0	0	0	10
Maximum	30	810	10	250	20
Mean	16	166	2	60	14
Dilution 1:100					
Minimum	0	Too few	0	Too few	0
Maximum	0	Too few	300	Too few	2,000
Mean	0	Too few	120	Too few	600
Sample G—One-Gram Portions					
Dilution 0					
Minimum	300	300	400	350	450
Maximum	650	700	700	500	1,150
Mean	480	500	540	450	810
Dilution 1:10					
Minimum	0	Too few	100	Too few	500
Maximum	1,500	Too few	150	Too few	3,000
Mean	700	Too few	90	Too few	1,400
Sample G—Five-Gram Portions					
Dilution 0					
Minimum	190	300	200	430	160
Maximum	380	560	280	660	470
Mean	296	432	245	590	342
Dilution 1:100					
Minimum	1,000	Too few	100	Too few	1,000
Maximum	2,000	Too few	150	Too few	7,000
Mean	1,400	Too few	90	Too few	3,400

difficulty experienced in obtaining absolutely uniform samples of gelatin and in view of the numerous factors that affect the total count.

On the basis of the results obtained, therefore, it cannot be concluded that five-per cent solutions of gelatin give more accurate or reliable bacterial counts than one-per cent solutions or vice versa. It is necessary to consider other factors that have come to light in this study before formulating a proper judgment in this matter. On this basis, there is little doubt that the one-per cent solutions of gelatin are preferable to the five-per cent solutions for the following reasons. The five-per cent solutions in zero dilution frequently give total counts that are too high to enumerate with accuracy if the count is in the range of 10,000 per gram or more, and the 1:100 dilutions of the same gelatins introduce an error based on the dilution factor which lessens the reliability of the final result.

It was also observed that the five-per cent solutions of gelatin frequently yielded spreaders and sporeformers which make accurate counting almost impossible. This was not the case with the same degree of frequency when one-per cent solutions were employed.

The preparation of five-per cent solutions of gelatin is attended with far more difficulty than the preparation of one-per cent solutions of gelatin. In five-per cent solution the gelatin often becomes lumpy and difficult to dissolve. It is also necessary to store such solutions at 45°C. for long periods of time during which microbic changes of appreciable significance may occur. The five-ml. portions in the Petri dishes often congeal before the warm nutrient agar is added. This increases the difficulty of obtaining reliable counts. These difficulties are entirely absent when one-per cent solutions are employed.

The five-per cent solutions of gelatin are much more viscous than the one-per cent solutions. Hence it is easier to introduce errors in quantitative measurements when five-per cent solutions are employed than when one-per cent solutions are used. This difficulty is also largely absent when one-per cent solutions are employed.

The use of five-per cent solutions of gelatin necessitates much more equipment and culture media and a material increase in time than does the use of one-per cent solutions of gelatin. This increases the cost of the bacteriological examination materially. However, this would be a secondary consideration if the use of the five-per cent solution enhanced the accuracy, reliability, or value of the bacteriological results. As this is not the case and as the accuracy and reliability of the counts made on the one-per cent solutions are probably greater than those made on the five-per cent solutions, it would seem that the use of the one-per cent solution is preferable.

In viewing this whole subject it is necessary to keep in mind the purpose of the bacteriological examination of gelatin. Gelatin is examined bacteriologically in order to determine the wholesomeness and the sanitary quality of the final product. The method of examination used should be simple, direct, and as accurate as possible. If a standard bacterial count for clean, wholesome gelatin is 10,000 per gram, it is going to make very little difference whether the count falls in the 9,000 group or the 8,000 group or lower. A one-per cent solution of such gelatin would give a total number of colonies on the plate which could be counted very easily and

accurately. An examination of the zero dilution of a five-per cent solution of the same gelatin would increase materially the difficulties of obtaining an accurate count.

Too much emphasis is placed on the significance of total counts in the sanitary bacteriological examination. In the lay mind a product reported as containing 9,000 bacteria per gram is considered superior to one reported containing 10,000 bacteria per gram. The pitfalls of the sanitary bacteriological examination are unknown or unrecognized. There is also widespread ignorance of the lack of statistical significance of minor variations in total counts. Unfortunately this condition has been fostered by the necessity for quantitative bacterial standards for public-health regulatory purposes and the strict enforcement of such standards by the legal authorities. In view of the many shortcomings of the quantitative bacteriological examination and the lack of statistical significance for appreciable variations in total count, the method of analysis adopted should be simple, accurate, and reliable. The theoretical attainment of the last microorganism present should not be the excuse for making the method of analysis difficult, costly, and complicated.

CONCLUSION

On the basis of statistical results obtained, as well as the items enumerated in the discussion, it is the opinion of this committee that the use of one-per cent solutions of 30- to 40-mesh gelatins is preferable to five-per cent solutions of such gelatins for the bacteriological examination. In the examination of coarse-ground gelatins (six to 10 mesh) or flake gelatins, five- or 10-per cent solutions will probably yield more reliable results. This study did not compile any data on the two latter types of gelatin.

EXTRACTS FROM IRISH MOSS AS A SUBSTITUTE FOR AGAR IN BACTERIOLOGICAL CULTURE MEDIA [1]

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Owing to the shortage of agar it is probable that some substitute will have to be used as a solidifying agent in bacteriological culture media. A possible substitute is carrageen,[2] a gelatinous substance extracted from a marine alga, *Chondrus crispus*, commonly called Irish moss. The similarity of carrageen to agar as to its origin and certain chemical and physical properties suggests it as a possible substitute for agar.

The purpose of this investigation is to determine first, if carrageen has the physical properties necessary for bacteriological culture media, and second, its effect, if any, on the growth cultural characteristics and the biochemical and antigenic properties of the microorganisms.

METHOD OF MAKING NUTRIENT CARRAGAR AND CONTROL AGAR

Plain nutrient broth was made, consisting of one per cent of peptone, five-tenths of one per cent of sodium chloride, and meat infusion. This mixture was heated until the ingredients were in solution, and the reaction was then adjusted to pH 7, and the mixture was allowed to cool to room temperature. This broth was divided into two portions. To one portion the desired amount of Carragar was added, and to the other portion was added an equal amount of agar to serve as a control medium. These were heated in a double boiler until they were in solution and the reaction was readjusted to pH 7 if necessary. They were then filtered through a thin layer of cotton, tubed, and sterilized in the autoclave at 15 pounds pressure for 15 minutes.

Carragar at high temperature is hydrolyzed in the presence of acids or alkalies; therefore the medium was neutralized before heating. It was found that Carragar goes into solution better if it is dusted on the surface of the liquid a little at a time and stirred well after each addition. If added in large quantities or to a hot liquid it forms lumps that go into solution slowly. Carragar filters more slowly than agar; it is therefore necessary that the medium be kept very hot during filtration. Two per cent was found to be the best concentration of Carragar for most purposes.

This nutrient Carragar served as a basis for all the special media used except for the synthetic medium. Beef extract and water can be substituted for meat infusion if desired.

Sodium chloride was added because it is a usual constituent of nutrient agar. Its presence in Carragar also causes a somewhat firmer gel.

[1] This investigation was supported by a grant from the Krim-Ko Company of Chicago, Illinois.

[2] The carrageen used in this investigation was a purified extract of *Chondrus crispus* made by this company and will hereafter be referred to as Carragar, the name given the product by the manufacturers.

CHEMISTRY OF CARRAGEEN

Extracts of *Chondrus crispus* are chiefly carbohydrate in nature. Haas and Hill (1921) separated the carbohydrate into cold-water and hot-water fractions. The hot-water fraction was found to consist of calcium salt of ethereal sulphate. The calcium was freely ionized but not the sulphate, unless the material was hydrolyzed. Butler (1936) considers that the extracts of *Chondrus crispus* represent not a single carbohydrate but a mixture of several carbohydrates of the ester and acid types.

$$(R[O \cdot SO_2 \cdot O]_2Ca)N$$
$$([RO \cdot SO_2 \cdot O]_2Ca)N$$
$$(HO \cdot SO_2 \cdot O \cdot R[O \cdot SO_2 \cdot O]_2Ca)N$$

What carbohydrates compose this polysaccharide is not well understood. Tollens (1914) considers it to be of the fructosan type with a certain number of galactose groups. Sabor (1900), however, demonstrated the presence of galactose, glucose, and fructose together with some pentosan and methyl pentosan. The percentage of inorganic matter is high. Butler (1934) found 20 per cent in the ash of the purified material. This ash is composed largely of calcium and potassium as salts of sulphates, chlorides, and phosphates. These salts are necessary for proper jellification.

PHYSICAL PROPERTIES OF CARRAGAR

The chief physical property of Carragar, like that of agar, is its ability to form a gel. The gel, however, differs in certain respects from that of agar: (1) Carragar gel is not so firm; (2) the melting point of Carragar gel is much lower; (3) Carragar is more easily hydrolyzed by hot acids and alkalies. To determine if the Carragar gel is firm enough for the different bacteriological procedures, nutrient culture media were made, having the following concentrations of Carragar: 1.5, 2.0, 2.5, and 3.0 per cent. As low a concentration of Carragar as 1.5 per cent was found to be firm enough to support itself in a thin or thick layer in an inverted Petri dish at a temperature of 37.5°C.(99.5°F.). It is therefore suitable for pour plates, stab cultures, and for semisolid media. For surface inoculations Carragar did not serve well, as the surface of the medium is too soft to inoculate in this manner without cutting or breaking the surface. Even three per cent of Carragar is not firm enough to be easily inoculated in this way. If, however, .5 to .75 per cent of agar is added to two per cent of Carragar a more solid gel is formed and one firm enough for surface inoculation.

The melting point of Carragar is about 45°C.(113°F.) and is high enough for cultivation of such organisms as grow at a temperature of 37.5°C., or lower. For the growth of the thermophylic type of organisms or conditions where it is necessary to maintain a temperature of 43°C. (109.4°F.) or above, Carragar will not serve. Here again the addition of agar will overcome this difficulty, for .5 per cent will raise the temperature of the melting point of the mixture to 68°C.(154.4°F.).

To determine the effect of acid and alkali on Carragar at autoclave temperature, 121°C.(250°F.), a nutrient medium was made containing

1.5 per cent Carragar. After filtration the medium was divided into eight equal portions. Each portion was adjusted to a different reaction pH, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, tubed in 10-ml. lots, and cooled to room temperature, 22°C. (71.6°F.). The media of all the different reactions were solid. After autoclaving at 15 pounds pressure for 15 minutes the medium with a reaction of pH 5 would not solidify on cooling.

The tubes of Carragar media of different reactions were then heated slowly in a water bath and the temperature noted at which the media softened and when it was liquid.

The results shown (Table 1) indicate that the range of the reaction at which it is safe to heat Carragar is narrow; therefore before cooking or sterilizing Carragar the reaction should be adjusted to near the neutral point.

When an acid or alkali medium is required, sterile acid or alkali can be added to the sterilized medium cooled to between 50 and 60°C. (122 and 140°F.). By this method media with a pH as low as 4.8 and as high as 9 were made without any sign of hydrolysis of the Carragar.

TABLE 1

Effect of Hot Acid and Alkali on Carragar

pH 5.0		pH 5.5		pH 6.0		pH 6.5		pH 7.0		pH 7.5		pH 8.0		pH 8.5	
Temp °C that medium softens	Temp °C that medium is liquid														
20	20	40	43	43	45	45	47	45	47	45	47	44	46	43	46

Fermentable Substance in Carragar: The following organisms were inoculated into plain nutrient 1.5 per cent Carragar containing Andradi indicator to determine if there was any fermentable carbohydrate present: *Staphylococcus aureus*, *Streptococcus fecalis*, *Shigella dysenteriae*, *Shigella paradysenteriae* (Flexner), *Shigella paradysenteriae* (Sonne), *Eberthella typhi*, *Salmonella paratyphi*, *Salmonella schottmülleri*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus polymyxa*, *Bacillus macerans*, *Corynebacterium diphtheriae*, *Vibrio proteus*, *Aerobacter aerogenes*, and an organism of the *Mucosus capsulatus* group. The only bacteria that gave any fermentation reaction in the Carragar were *Aerobacter aerogenes* and the organism of the *Mucosus capsulatus* group.

The controls of plain nutrient agar were all negative. These results indicate that Carragar contains some substance, either in itself or as an impurity, that is fermentable by some bacteria. The presence of this substance may to some extent interfere in the study of the carbohydrate fermentation by bacteria. It is possible, however, that this difficulty can be overcome by the proper use of controls and a more complete knowledge of what organisms ferment it.

TABLE 2

Comparison of Growth of Organisms on Carragar and Agar Media After 24 and 48 Hours of Incubation[1]

Organism	Plain Carragar		Plain agar		Glucose Carragar		Glucose agar		Blood Carragar		Blood agar	
	24	48	24	48	24	48	24	48	24	48	24	48
Staphylococcus aureus	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Streptococcus fecalis	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Streptococcus hemolyticus	—	+	—	—	+++	+++	+++	+++	++++	++++	++++	++++
Streptococcus viridans	—	—	—	—	+++	+++	+++	+++	++++	++++	++++	++++
Pneumococcus I	—	—	—	—	++	++	++	++	++++	++++	++++	++++
Pneumococcus III	—	—	—	—	+	+	—	+	++++	++++	++++	++++
Gonococcus	—	—	—	—	—	—	—	—	+++	+++	+++	+++
Meningococcus	+	+	—	—	+++	+++	++	++	....	....	....	....
Sarcina lutea	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Shigella dysenteriae	++++	++++	+++	+++	++++	++++	++++	++++	....	....	....	....
Shigella paradysenteriae (Flexner)	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Eberthella typhi	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Salmonella paratyphi	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Salmonella schottmülleri	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Escherichia coli	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Aerobacter aerogenes	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Proteus vulgaris	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Bacillus abortus	+++	++++	++	++++	+++	++++	++	+++	....	....	....	....
Pseudomonas aeruginosa	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Serratia marcescens	++	++++	++	++++	++	++++	++	++++	....	....	....	....
Bacillus subtilis	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Bacillus mesentericus	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Corynebacterium diphtheriae	+	+	+	+	+++	+++	+++	+++	....	....	....	....
Micrococcus smegatis	+	+++	+	+	+	+++	+	+++	....	....	....	....
Clostridium tetani	—	+	—	+	+	+++	+	+++	....	....	....	....
Clostridium welchii	—	—	—	—	+++	+++	+++	+++	....	....	....	....
Vibrio proteus	++++	++++	++++	++++	++++	++++	++++	++++	....	....	....	....
Leptothrix	+	+++	+	+++	++	+++	++	+++	....	....	....	....
Yeast	+	++	+	+	+++	+++	+++	+++	....	....	....	....
Tricophyton	+	—	—	—	....	+++	....	+++	5 days	....	....	....

[1] ++++ = excellent growth; +++ = good growth; ++ = fair growth; + = poor growth; — = no growth.

COMPARISON OF GROWTH OF ORGANISMS ON CARRAGAR AND AGAR

To determine the ability of Carragar to support growth of organisms as compared with agar, both were inoculated and the amount of growth observed after 24 and 48 hours of incubation at 37°C.(98.6°F.). The organisms used were selected to represent a number of groups or types.

The results (Table 2) indicate that the organisms used in all cases grew as well on Carragar as on agar and in some cases even better. The meningococcus, for instance, grew on plain Carragar and failed to develop on plain agar. *S. dysenteriae* and *B. abortus* grew better on Carragar. By this method, unless the difference in the amount of growth is relatively great,

TABLE 3

Comparison of Number of Colonies Developing on Carragar and Agar After 24 and 48 Hours of Incubation

Organism	Plain Carragar		Plain agar		Glucose Carragar		Glucose agar	
	24	48	24	48	24	48	24	48
Staphylococcus aureus	168	172	108	142	181	189	160	184
Streptococcus fecalis	112	116	101	120	121	132	105	115
Meningococcus	12	16	0	0	32	38	20	29
Sarcina lutea	196	195	183	190	200	204	188	197
Shigella dysenteriae	116	124	104	120	128	130	112	120
Shigella paradysenteriae (Flexner)	160	170	140	152	165	168	150	160
Eberthella typhi	164	Spreader	142	158	173	Spreader	160	175
Salmonella paratyphi	232	243	216	230	248	256	228	240
Salmonella schottmulleri	286	297	249	260	299	305	271	286
Escherichia coli	272	281	246	259	291	298	260	281
Aerobacter aerogenes	260	272	236	263	282	289	245	266
Bacillus abortus	96	115	80	107	99	112	89	105
Pseudomonas aeruginosa	231	240	239	252	228	239	235	261
Bacillus subtilis	188	201	146	168	181	190	160	178
Corynebacterium diphtheriae	96	102	79	92	92	98	84	90
Vibrio proteus	280	294	267	286	278	286	270	289
Leptothrix	63	82	51	79	70	72	58	78
Yeast	72	78	60	72	89	96	61	84

it is not easily detected. Therefore to determine this point more accurately the following method was used:

A 24-hour broth culture of each organism was diluted with sterile broth so that one ml. would contain from one to three thousand organisms. One-tenth ml. was delivered into a sterile Petri dish, the melted medium added, the two were mixed, allowed to harden, and were incubated at 37.5°C.(99.5°F.). A count of the number of colonies appearing in the plates was made at the end of the 24 and 48 hours' incubation. The plates were made in duplicate and the experiment carried out three times; therefore, the figures (Table 3) represent the average number of organisms on six plates.

More colonies developed on plain and glucose Carragar than on plain and glucose agar with the exception of *Ps. aeruginosa* on plain and glucose agar and *Vib. proteus* and Leptothrix on glucose agar.

VIABILITY OF ORGANISMS ON CARRAGAR AND AGAR

The viability of organisms on Carragar as compared with agar was tested by inoculating the organism on Carragar slants and on agar slants. After 24 hours of incubation at 37.5°C. the cultures were kept at room temperature, and at weekly intervals subcultures were made in duplicate. The subcultures were made on glucose agar slants for all except those on blood medium. The gonococcus and meningococcus were kept at 37.5°C. and subcultured daily. The streptococcus and pneumococcus were transferred at the end of the first week and every second day thereafter.

A slight difference is noticeable in survival-time of the organisms tested in favor of Carragar (Table 4).

COLONY CHARACTERISTICS

To determine the effect of Carragar on colony characteristics the following organisms were inoculated on plain Carragar and plain agar slants:

TABLE 4

Comparison of Viability of Organisms on Carragar and Agar

Staphylococcus aureus	*Staphylococcus fecalis*	*Sarcina lutea*	*Shigella dysenteriae*	*Shigella paradysenteriae*	*Eberthella typhi*	*Salmonella paratyphi*	*Salmonella schottmulleri*	*Escherichia coli*	*Aerobacter aerogenes*	*Bacillus proteus*	*Bacillus abortus*	*Pseudomonas aeruginosa*	*Corynebacterium diphtheriae*	*Micrococcus smegatis*	*Micrococcus subtilis*	*Vibrio cholera*	*Streptococcus hemolyticus*	Pneumococcus Type I	Gonococcus	Meningococcus
Carragar[1]																				
A	49	A	35	42	49	49	56	70	70	70	35	70	35	A	A	49	31	21	6	10
							Agar										Blood Agar			
A	49	A	35	42	42	49	56	63	70	70	42	63	35	A	A	49	27	21	4	7

[1] Figures = number of days organisms remained viable; A = organisms still viable at 77 days.

Staph. aureus, S. dysenteriae, E. typhi, E. coli, A. aerogenes, B. abortus, B. subtilis, and *Vib. cholera.* The meningococcus was inoculated on glucose Carragar and glucose agar. The organisms were transferred to fresh media every second day for eight days. On the ninth day suspensions of the organisms were streaked on plates, the Carragar culture onto Carragar and agar plates and the agar culture onto agar and Carragar plates. These plates were incubated 24 hours and examined.

Well-separated colonies were larger on Carragar than equally well-spaced colonies on agar. With colonies close together there was a greater tendency for them to coalesce on Carragar.

Spreading types of colonies were more common on Carragar plates. This is probably due to the greater amount of moisture in the Carragar gel. *Staph. aureus* had about 10 per cent more white colonies on the Carragar plates than on the agar plates. Other than this there was little evidence of any change in the colony characteristics.

MORPHOLOGY

Smears were made from the slants and separate colonies from the plates of the organisms used in the experiment on colony forms. These smears were stained with methylene blue and by Gram's method. The only organism that showed any change in morphology was the meningococcus. These organisms were much larger and many more tetrads were present on the Carragar cultures.

BLOOD CARRAGAR

Blood may be added to nutrient Carragar if certain precautions are taken, for blood causes a rapid solidification of Carragar. The larger the amount of blood added the more difficult it is to get an even mixture. Ten per cent of blood may be added without difficulty. Whole blood, defibrinated, or citrated blood may be used. The following method of preparation gave the best results:

Use 1.5 per cent nutrient agar. Keep the melted Carragar at 50°C. (122°F.). Warm the blood to 50°C., add it *immediately,* and mix *thoroughly.* Once mixed there will be no separation of the two or solidification of the medium so long as the temperature is kept at 50°C. It is well to warm the Petri dishes; if they are cold the medium may solidify before it spreads evenly over the surface of the dish. It is necessary to add .5 per cent sodium chloride to the nutrient Carragar when it is to be used for blood medium. While Carragar has a higher salt content than agar it is not sufficiently high to make the medium isotonic with the blood and prevent hemolysis of the cells. The addition of .5 to .75 per cent agar to the Carragar gave a better blood medium both for plates and slants as the surface is firmer and easier to inoculate.

Growth of Streptococcus and Pneumococcus in Blood Carragar: Blood Carragar was inoculated with *Strep. hemolyticus* and *Strep. viridans* and their growth compared with that on blood agar. Both types of streptococci grew as well on Carragar and there was no apparent change in the colony characteristics or morphology. *Strep. hemolyticus* had a wide, clear zone of hemolysis around the colonies on the blood Carragar plate equal to that on the blood agar plates. *Strep. viridans* gave the green halo around the colonies on both media

Streptococcus hemolyticus was isolated equally as well on blood Carragar as on blood agar from a case of bronchopneumonia.

Pneumococci were isolated from a pneumonic sputum on blood Carragar and on blood agar. The organism, as determined by the Neufeld Quellung reaction using the sputum, was Type 29. The pneumococcus colonies on the blood Carragar were typical and more abundant than on blood agar. The Neufeld Quellung reaction and the agglutination test were performed on the pneumococci isolated on both kinds of media. The Quellung reaction was positive with the antipneumococcus serum Type 29. The capsules were distinct and enlarged in the cultures from both the blood Carragar and the blood agar. The agglutination test was equally satisfactory, the pneumococci being well clumped in cultures from both media. Pneumococcus Types I and III from stock cultures were grown on blood Carragar for several generations without any apparent change in its normal morphology and colony form.

ENDO'S MEDIUM

Endo's plates require a dry, firm surface for the best results; therefore, Carragar alone did not serve well for this purpose. When however, .75 per cent of agar was added to two per cent Carragar the mixture served very well. The reagents mixed well with the medium and the colonies were well defined. The lactose-fermenting organisms produced the characteristic red colonies and *B. coli* had, in addition, the typical metallic sheen. The typhoid and the paratyphoid organisms and the Shiga and Flexner types of the dysentery bacilli produced the typical colorless colonies on Carragar that they do on agar.

CHOCOLATE MEDIUM

Carragar may be used in the place of agar in making chocolate medium. The blood must be quickly mixed with Carragar, although this is not as important as it is in the making of blood medium, for in making chocolate medium the Carragar can be used at a higher temperature and therefore with less danger of a too-rapid solidification of the medium.

Stock cultures of the gonococcus and the meningococcus grew well on this medium.

TABLE 5

Comparative Count of Colonies Developing on Carragar and Agar From Samples of Water

Sample No.	Incubated at 20°C. for 48 hr.				Incubated at 37.5°C. for 24 hr.			
	Carragar plates		Agar plates		Carragar plates		Agar plates	
1	220	232	160	153	Spreader	204	165	181
2	228	216	140	180	209	212	150	154
3	260	248	201	185	182	Spreader	192	205
4	176	135	134	142	168	172	145	152
5	163	166	136	131	152	165	126	135

SYNTHETIC MEDIUM

A synthetic medium was made consisting of asparagin and sodium lactate as the source of nitrogen and carbon, buffered with disodium hydrogen phosphate and monopotassium hydrogen phosphate with a trace of calcium and magnesium, with Carragar as the solidifying agent. The organisms tested grew as well on this Carragar synthetic medium as they did on the same medium solidified with agar.

WATER PLATES

Five separate samples of water were plated on Carragar and on agar. Two sets of plates were made in duplicate from each sample, and one set was incubated at 20°C. (68°F.) for 48 hours; the other set was incubated at 37.5°C. (99.5°F.) for 24 hours. The number of colonies were then counted.

The number of colonies developing on Carragar was greater than that developing on agar at both temperatures of incubation (Table 5). Two of the Carragar plates incubated at 37.5°C. had a spreading type of colony.

MILK PLATES

Samples of milk were plated on Carragar and on agar and incubated at 37.5°C. for 24 hours and at 20°C. for 48 hours. The number of colonies

developing on the Carragar plates at both temperatures of incubation was greater than the number of colonies on the corresponding agar plates.

PIGMENT FORMATION

The chromogenic bacteria produce their characteristic color on Carragar but there was some variation as to intensity as compared with agar. On Carragar the pigment was better in cultures of *Ps. aeruginosa,* and in *M. smegatis* there was a dark yellow pigment not seen on agar. *Staph. aureus* and *S. marcescens* produced a more intense color on agar. Pigment formation by many of that type of bacteria is a variable characteristic, therefore difference in the amount of pigment produced in the two media is of little significance. The only important fact is that the color was produced on Carragar by these pigmented organisms.

AGGLUTINATION

Agglutination tests were made with *E. typhi* and the Shiga and Flexner types of *S. dysenteriae*. The organisms used had been growing on Carragar for 60 days with four intervening transfers. The controls were the same bacteria that had grown continuously on agar. Twenty-four-hour cultures of the organisms on the two media were washed off from the slants with sterile broth, and macroscopic agglutination tests were set up in the usual manner. The titer was the same with the organisms grown on Carragar as those grown on agar.

SUMMARY

Extracts of *Chondrus crispus* can be used as a solidifying agent in bacteriological culture media. It differs from agar in some of its physical properties. The gel is not as firm as that formed by agar. The melting point (45°C.) is much lower than that of agar. It is more easily hydrolyzed by hot acids or alkalies. The gel, however, is firm enough for most purposes and where a firmer gel is needed the addition of .5 to .75 per cent of agar will overcome this difficulty. The addition of agar also raises the melting point so that incubation at high temperatures is possible. The neutralization of the Carragar medium before heating will prevent its hydrolyzation.

Carragar served well in all the different kinds of culture media tried. Growth of the different organisms used was as good on Carragar as on agar and in many instances it seemed to be better, and the viability of the organism was as great. There was no change in the colony characteristics or morphology with the exception of a slight one in the Meningococcus, and no change in the biochemical or antigenic properties of any of the organisms growing on Carragar.

Samples from different lots of Carragar were used and were quite uniform in their properties.

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THIAMINE DETERMINATION BY THE FUNGUS-GROWTH METHOD AND ITS COMPARISON WITH OTHER METHODS[1]

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The basis of the fungus-assay method for thiamine determination lies in the fact that growth of the fungus, *Phycomyces blakesleeanus*, on a mineral-dextrose medium containing asparagine, is proportional to the amount of thiamine added with additions of small amounts of the vitamin. A comparison of the amount of growth produced through the additions of known amounts of thiamine with that produced by the addition of specific amounts of the material whose thiamine content is unknown provides a basis of estimation of the vitamin content of the unknown.

The use of *Phycomyces blakesleeanus,* in a quantitative biological assay for thiamine was proposed by Schopfer and Jung (1937). Since then, several investigators—Bonner and Erickson (1938), Burkholder and McVeigh (1940a), Burkholder and McVeigh (1940b), Sinclair (1938), and Villela (1938)—have studied the method. Although it has not come into widespread usage, for certain types of work, it presents advantages over all other available methods. In routine analysis of large numbers of samples, it has given us the maximum results with the minimum of labor and expense. This laboratory has initiated a study of the factors influencing the nutritive value of foods crops—Hamner, Lyon, and Hamner (1942) and Ellis and Hamner (1943)—and a large number of samples of tomatoes and wheat have been available for thiamine analysis. The fungus method has been found accurate for these materials on the basis of a comparison with the rat-curative assay, and in our experience analyses can be made more efficiently by it than by any other of the available techniques. Analyses of a variety of vegetable samples have shown close agreement between the fungus-assay and the thiochrome method.

The purpose of this paper is two-fold: first, to present data which indicate that this assay method may give results in agreement with those obtained by the rat-assay and thiochrome methods; and second, to show that consistent results are obtained in repeated analysis of the same material. Details of procedure are included, since these represent modifications of published techniques recommended to save labor in the analysis of large numbers of samples.

EXPERIMENTAL METHOD

The composition of the basal thiamine-free nutrient medium is shown (Table 1). Twenty-five ml. of this medium are added to each of a requisite number of 125-ml. Erlenmeyer flasks. To some of the flasks known amounts of pure thiamine hydrochloride (Merck) are added to give a series in

[1] The authors gratefully acknowledge the suggestions of Dr. L. A. Maynard.

which the thiamine content varies from .1 to .6 microgram in increments of .1 microgram. To other flasks are added sufficient amounts of the various samples to be analyzed to supply between .1 and .6 microgram of thiamine. The flasks are stoppered with cotton plugs and are sterilized in an autoclave at 15 pounds for 15 minutes and allowed to cool. Stock cultures of *Phycomyces blakesleeanus* are grown for two or three weeks in test tubes on agar slants on a medium made by the addition of two per cent agar, .1 per cent d'Witte's peptone, and approximately one microgram of thiamine per 100 c.c. to the basal nutrient medium. A spore suspension is made by shaking the spores from one or two of the tubes of stock culture in 25 ml. of sterile distilled water. Each of the culture flasks is inoculated with one drop of the spore suspension by means of a sterile pipette. Inoculation is made within two hours after the spore suspension has been prepared. After inoculation, the culture flasks are kept at a temperature of approximately 24°C.(75.2°F.) for two weeks. At the end of this time, the mycelial mats are transferred from the flasks to a Büchner funnel lined with a hard filter paper and washed very thoroughly with

TABLE 1

Composition of Basal Thiamin-Free Nutrient Solution

Water	1,000 ml.
Asparagine	5 gm.
Dextrose	50 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
KH_2PO_4	1.5 gm.
Ferric citrate (.1 per cent solution)	0.22 ml.
Microelement solution [1]	0.1 ml.[1]

[1] Each liter of this solution contains the following salts: $ZnSO_4$— 245 gm., $CuSO_4$—.0502 gm., $MnSO_4$—.0275 gm., H_3BO_4— 0572 gm., $(NH_4)_2MoO_4$—.0327 gm.

distilled water. The mat is then rolled into a ball with the fingers, placed on a wooden tray ruled off into numbered squares, and transferred to a drying oven at 100°C.(212°F.). After drying for 24 to 48 hours, the individual mycelia are removed from the oven and allowed to cool and to come to equilibrium with the moisture of the atmosphere. The mats are then weighed to a tenth of a milligram. The amount of growth obtained in the flasks containing known amounts of thiamine is used to prepare a standard curve showing the amount of fungus growth per unit of thiamine. The amount of growth obtained in the samples being analyzed is compared to this standard curve to indicate the amount of thiamine present in the unknown sample.

DETAILS OF PROCEDURE

Preliminary Tests: When working for the first time with any particular type of material, certain preliminary determinations should be made before proceeding with quantitative assays. First, it is necessary to know approximately the amount of thiamine present in the material in order to adjust the sample size for the quantitative determination to contain between .1 and .6 microgram. This range may be found by testing a series of samples over a wide concentration range, using ten-fold dilutions. Second, it is necessary to know if toxic or growth-inhibiting substances

are present in the material. This may be determined as follows: After preparing an extract of the sample for analysis, it is added to a series of culture flasks in aliquots adjusted to contain from approximately .1 to .6 microgram of thiamine. If materials decreasing growth are present, the growth increments, resulting from increasing amounts of the unknown, will not be as large as the growth increments resulting from increasing amounts of pure thiamine. In other words, reference to the standard-growth curve (based on the addition of pure thiamine to the basal media) will indicate successively lower readings for the thiamine content of the unknown as increasing aliquots are used. Third, it is known that the two vitamin intermediates, the thiazole and pyrimidine moieties, if both are present in the sample, stimulate the growth of the fungus in much the same manner as does the vitamin itself and will lead to an erroneously high reading. The possible presence of these substances should be determined by comparing the results obtained by the fungus-assay method with those obtained by some of the other available methods. The sulfite-cleavage method of Schultz, Atkin, and Frey (1942) may also be used to test for the presence of these substances. Work here has indicated that the fungus method is satisfactory for the routine analysis of several types of plant material. It is believed that it may be found accurate with many other types of material.

Preparation of Culture Flasks: The Erlenmeyer flasks used in culturing the fungus should be thoroughly cleaned, since the amount of thiamine necessary to produce an appreciable amount of growth of the fungus is quite small. The use of sulfuric acid cleaning solution was found to remove apparently all traces of thiamine. The addition of 25 ml. of the basal nutrient to each of several hundred flasks is a laborious task. However, since the amount added need not be exactly 25 ml. but may vary by a few milliliters without materially affecting the results, it is convenient to use a 25-ml. pipette with a broken tip which allows rapid drainage. Another technique which saves even more time in the addition of basal nutrient is to utilize a syringe, graduated to deliver 25 ml. when the plunger is fully extended. With either procedure it is essential that none of the basal nutrient be allowed to touch the neck of the flask, since if this happens, the cotton plugs stick to the glass and are difficult to remove, thus increasing chances of contamination. After the addition of the basal nutrient, extracts of the samples to be analyzed are pipetted into the flasks and cotton plugs inserted. From four to six replicate flasks should be used for each extract to minimize the error of the determination. The flasks are then autoclaved at 15 pounds for 15 minutes and are ready for inoculation.

Asparagine: Asparagine as obtained from the commercial supply houses contains an appreciable amount of thiamine and should be purified. This may be done by dissolving it in hot water to form a nearly saturated solution, adding a little charcoal, and filtering while hot. As the filtrate cools, the asparagine will crystallize. One subsequent recrystallization is usually sufficient to give a very pure product. At present asparagine is very expensive and is difficult to obtain. Burkholder and McVeigh (1940a) have indicated the possibility of using other sources of nitrogen satisfactorily, thus eliminating the necessity of asparagine.

Spore Suspension: Bonner and Erickson (1928) have pointed out that a difference of a thousandfold in the number of spores used in the inoculum may make very little difference in the final result. It is preferable to use a concentration of spores that is near the minimum necessary to insure establishment of the organism, since if too heavy a suspension is made, it is likely to contain amounts of thiamine sufficient to produce erroneous results. A proper suspension should result in fungal growth in all cultures, but those containing only the thiamine-free basal solution should result in growth less than one mg. The suspension is made by shaking some of the sporulating mycelium of a stock culture in sterile distilled water. None of the substrate should be included in this process, since to do so would add thiamine to the suspension.

Preparation of Extract: A thoroughly ground sample of fresh or dried material of suitable size is weighed and added to a 300-ml. Erlenmeyer flask containing 75 ml. of N/10 sulfuric acid. The flask is placed in a boiling water bath for 30 minutes and allowed to cool. The resulting extract containing the sample in suspension is neutralized with one normal KOH with constant stirring to a pH between 4 and 5. In routine work, it is possible to adjust the concentration of the KOH solution so that the addition of just 10 ml. of the alkali will result in the desired pH with a great saving in time. The resultant suspension is made up to a volume of 100 c.c. and filtered, or the solid material is simply allowed to settle. An aliquot of the filtrate or of the supernatant liquid is added directly to the culture flasks. The amount of aliquot used should be sufficient to supply from .15 to .5 microgram of thiamine to the culture flasks. The volume of the aliquot should not be greater than four ml. If such an aliquot contains less than .15 microgram of thiamine, another extract should be made which contains a larger amount of the original sample.

Incubation: In these experiments the cultures were incubated for two weeks at 24°C.(75.2°F.). While this temperature is somewhat higher than that recommended by others, it has given satisfactory results. Since this is above usual room temperature, the maintenance of uniform temperatures in the incubator is not difficult. A discussion of the effects of temperature upon the growth of Phycomyces may be found in the paper by Burkholder and McVeigh (1940a). Some investigators have grown their cultures for a shorter period than two weeks, but in our experience this longer period is desirable. Better agreement was consistently found between duplicate samples when two weeks' incubation was used rather than one week. Furthermore, determinations based on different sized aliquots of the same extract gave closer agreement with two weeks' incubation rather than one. The results obtained here indicated that little dry weight accumulation occurs during the second week of incubation and that in some cultures there may actually be a slight loss in weight. It appears, therefor, that the fungus in the various cultures grows rapidly until the thiamine is exhausted and then further growth ceases. Apparently, the dry weight produced is proportional to the total amount of thiamine in the culture rather than to the concentration, and thus the final result would be expected to be independent of growth rate. That such is the case is substantiated by the following experiment. A standard-growth curve was

prepared as described by adding varying amounts of pure thiamine to a series of cultures. Another series of cultures was prepared in exactly the same way, except that in addition to the thiamine a small amount of thiamine-free factors Z_1 and Z_2, described by Robbins and Hamner (1940), were added to each flask. The amounts of these factors added greatly stimulated the early growth of the cultures to which they were added, but the resulting dry weight after two weeks was the same for a given amount of thiamine whether or not the supplementary growth factors were added. If, as these results indicate, this method is independent of growth rate, then this fact may represent an advantage of this method over some of the other microbiological assays which are dependent upon growth rate.

Harvesting the Fungus: The description previously given of a method of harvesting the mycelial mats and determining their weight seems the most convenient for rapid work. The weighings should be done rapidly and on a day during which the atmospheric humidity does not fluctuate rapidly. If several hundred weighings are made during the day, it is well to reweigh some of the mats at the end of the day to determine the extent of the change in weight that may have occurred. In these experiments a Christian Becker balance of the projection reading type was used because of the rapidity with which a large number of weighings may be made. A Roller-Smith torsion balance permitting readings to .1 milligram has also been found satisfactory as well as an ordinary chain-o-matic balance, which permits fairly rapid weighings. The essential point is that, if the fungal mats are weighed while their moisture content is in equilibrium with atmospheric moisture, the weighings must be rapid enough so that changes in atmospheric humidity during the course of the day will not materially affect the final result.

DISCUSSION

The use of this method of thiamine assay must depend to a large extent upon its specificity. It is apparent that growth stimulation of the fungus is not a specific test for thiamine, since certain other substances will also stimulate its growth. It has been shown by Lilly and Leonian (1940), Schopfer (1939), and Villela (1938) that co-carboxylase is equally effective as thiamine in promoting the growth of the fungus. This is advantageous, since co-carboxylase, similar to thiamine, has vitamin activity for animals. Robbins and Kavanagh (1942), in a review of the literature in this field, list substances found to be effective as well as those not effective in replacing thiamine as a growth-promoting factor for *Phycomyces blakesleeanus.* Thiochrome, biotin, pyridoxine, riboflavin, nicotinic acid, inositol, and pantothenic acid have all been reported as noneffective. It was found here that ascorbic acid, choline hydrochloride, and indoleacetic acid were also noneffective. The pyrimidine and thiazole components of thiamine are each ineffective in themselves, but an equimolar mixture of them is as effective as the vitamin itself. Thus the presence of these two components in a sample to be analyzed by the fungus method will lead to an erroneously high reading for thiamine. This requires that for every type of material for which the method is to be used, it is necessary first to establish the validity of the assay by comparing it with some other assay procedure. However, once it is established that a particular type of material does not

contain interfering substances, it seems likely that the method may be used for this material satisfactorily. This has been found true for the samples of wheat and tomatoes reported here.

More than a thousand thiamine determinations have been made by the fungus method on samples of wheat and tomatoes. For these two materials a comparison has been made between results obtained by the fungus-growth assay and by the rat-curative assay (1939 Supplement U.S.P. XI). Assaying dried-tomato fruits by the rat-curative method, the aggregate-cure period of 17 rats on a level of 5.14 micrograms of pure thiamine was 164 days. The aggregate-cure period for these same rats on a level of .64 gram of dried tomato was 169 days. Thus, .64 gram of tomato contained 5.14 micrograms of thiamine or 8.0 micrograms of thiamine per gram. The thiamine content of dried tomato, according to the fungus-growth method, was 8.3 micrograms per gram or a difference between the methods of about 3.6 per cent.

Assaying ground wheat grains by the rat method, the aggregate-cure period of nine rats on a level of 4.71 micrograms of pure thiamine was 86 days. The same rats on a level of 1.1 grams of wheat were cured for a total period of 84 days. Thus, one gram of wheat contained 4.3 micrograms of thiamine. The thiamine content of wheat, according to the fungus method, was 4.7 micrograms per gram or a difference between the methods of about 8.5 per cent. Considering this relatively close agreement between the two methods, it seems safe to assume the fungus-growth method is a valid, accurate means of determining thiamine in tomato and wheat.

A comparison of analyses on vegetables obtained by the fungus method and by the chemical-assay method is illustrated (Table 2). These data have been taken from a paper by Heller, McCay, and Lyon (1943). The thiamine analyses of their samples were made by the fungus assay in this laboratory and comparable samples were analyzed for thiamine under the direction of Dr. L. R. Cerecedo of Fordham University, using a chemical method of assay, Hennessy (1942). The different samples of a given vegetable are not comparable to one another since samples from various sources, cooked and treated in a variety of ways, are represented. The agreement between the two methods (Table 2) on individual samples is remarkably close.

At the time the above analyses were made another set of analyses was made on food samples containing meat. In every case the fungus method gave values distinctly higher than those obtained by the chemical assay. The discrepancies between the two methods ranged from 30 to 100 per cent. The cause of this disagreement is not known, but it may be possible that these meat samples contained the vitamin components, the thiazole and pyrimidine moieties. More work must be done before the fungus method can be recommended for meats.

In the course of thiamine determinations on wheat preliminary to other extensive studies, results were obtained which illustrate that consistent values are obtained by the fungus-growth method. A large number of heads of the Nured variety, crop of 1939, were obtained from Dr. H. H. Love. Wheat from 10 heads was ground to give a composite sample and analyzed. The thiamine value was 5.17 ± .040 micrograms per gram of

wheat. The grain from each of 10 other heads of the same wheat was ground and analyzed for thiamine. The thiamine values, as micrograms per gram of wheat with standard error, were: 4.96 ± .103; 5.10 ± .049; 5.19 ± .132; 5.33 ± .045; 5.62 ± .118; 5.25 ± .063; 5.02 ± .046; 5.54 ± .065; 5.34 ± .069; 5.15 ± .020. Ten additional heads of the same wheat were selected and the grains removed from each head in order, beginning from the bottom. The three lowermost grains from the 10 heads were com-

TABLE 2

Comparison of Thiamine Values[1] *Obtained by Fungus and by Chemical Assays*

Material	Sample	Thiochrome[2] method	Fungus method
Potatoes	1[3]	0.90	0.71
	2	0.77	0.77
	3	0.70	0.69
	4	0.80	0.84
	5	0.83	0.88
	6	0.53	0.82
	7	0.76	0.80
	8	0.76	0.88
Peas	1	3.54	3.32
	2	2.62	2.64
	3	2.59	2.64
Spinach	1	0.86	1.01
	2	0.78	0.76
	3	0.67	0.78
Cole slaw	1	0.54	0.71
	2	0.48	0.57
	3	0.46	0.63
Carrots	1	0.70	0.82
	2	0.38	0.32
	3	0.42	0.40
Lima beans	1	1.00	0.79
	2	0.58	0.63
	3	0.85	0.50
	4	1.10	1.10
	5	0.62	0.75
	6	0.60	0.64
	7	0.93	0.85
	8	0.70	0.58
	9	0.55	0.54
Green beans	1	0.64	0.73
	2	0.47	0.56
	3	0.68	0.62
	4	0.70	0.86
	5	0.56	0.46
	6	0.46	0.47
	7	1.02	0.96
	8	0.76	0.67
	9	0.75	0.65

[1] Given in micrograms per gram, fresh weight. [2] Chemical analyses were made at Fordham University by M. Soodak and L. J. Vinsin, under the supervision of L. R. Cerecedo. [3] The samples as numbered differ as to origin and treatment and thus are not comparable with each other. They represent a part of a study on the influence of cooking on vitamin losses by Heller, McCay, and Lyon (1943). The significance here is in the agreement between the two methods on a given sample.

bined and ground to give a composite sample at this position in the head. The three next grains of each head were similarly combined and this was repeated, going up the head, to give a total of 11 samples. Thiamine values, as micrograms per gram of wheat, were (beginning with the sample corresponding to the grain in the lowermost position of the head and proceeding upward in order): 4.34 $\pm$ .069; 5.02 $\pm$ .088; 5.15 $\pm$ .074; 5.25 $\pm$ .062; 5.39 $\pm$ .014; 5.43 $\pm$ .085; 5.56 $\pm$ .022; 5.49 $\pm$ .101; 5.73 $\pm$ .077; 5.45 $\pm$ .129; 5.45 $\pm$ .045. These determinations were made in sextuplicate.

From the above data, the following mean values for the thiamine content of 10 heads are obtained (as micrograms per gram): ten heads composited 5.17 $\pm$ .040; 10 individual heads, 5.25 $\pm$ .067; and combining corresponding groups of three grains from 10 heads, 5.30 $\pm$ .036. Thus, the average vitamin content of 10 heads of this variety of wheat, as determined by three methods of sampling, agrees within the limits of error. Other thiamine analyses by the fungus-growth method made at intervals over a period of several months on the same sample of wheat have likewise given consistent results.

SUMMARY

This paper discusses a method of determining thiamine in plant material by measuring the growth of the fungus, *Phycomyces blakesleeanus,* on extracts of these materials. Details of procedure are given for adapting this method to the routine analysis of large numbers of samples. Comparing determinations by this method and by the rat-curative method, a difference of 3.6 per cent in thiamine content of tomato fruits and 8.5 per cent in wheat grains was found. In the analysis of various vegetable samples the fungus method has been found to agree closely with the thiochrome method. On meats, the former method gives consistently higher results. Thiamine determinations by the fungus-growth method on many samples of wheat indicate that it gives consistent results with a low error.

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CONTROL OF OXIDATIVE FLAVORS IN BEVERAGES [1]

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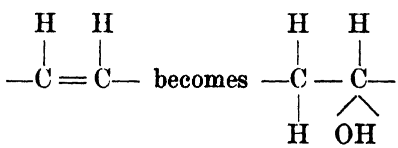
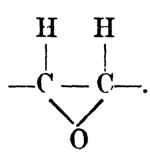
The entire food industry is rapidly becoming more and more concerned with the problem of development of oxidative "off" flavors in its products. Among the many products susceptible to these defects are fats, beer, milk, and fruit juice and other beverages.

Increasing concern over the problems of oxidized flavors has served to stimulate work on discovery of means for eliminating such defects. Incorporation of edible chemical compounds which will prevent or slow down the rate of oxidation of food products, so as to extend appreciably their shelf life, has been suggested and tried. Such chemical antioxidants to be usable must be cheap, harmless, and contribute no objectionable flavor of their own when added to any product.

There are two general theories as to the mechanism of oxidation of organic compounds. One is that oxidation is effected by direct addition of molecular oxygen to unsaturated linkages, while the other theory (rather generally accepted as the mechanism of biological oxidation, at least) is that oxidation takes place in two distinct steps: first, an unsaturated linkage is hydrated so that



and second, the two hydrogens of the added water are transferred to another reducible compound, either molecular oxygen or some other suitable hydrogen acceptor, the net effect of the two steps being that oxygen has been added to the molecule, leaving a group such as



Often, in aerobic biological oxidation, the hydrogen taken from the hydrated substrate being oxidized is transposed consecutively to four or five different intermediate carriers before the hydrogen reaches molecular

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oxygen, its final acceptor, to reform water. For example, suppose there exists in a system a molecule A to be oxidized, three intermediate carriers with progressively increasing oxidation potentials, B, C, and D, and the final hydrogen acceptor, oxygen, which will be called E. Under suitable conditions B will accept hydrogen from the hydrated form of A, thus oxidizing A and itself becoming reduced, but C, at a higher potential than B, in turn takes the hydrogen from B, thereby oxidizing B, and itself becoming reduced. But D is at a still higher potential and so takes the hydrogen from C, which is then changed back to its original state, D now existing in the reduced state. Finally oxygen may take the hydrogen from D, thereby reforming the water originally used to hydrate A, and restoring D to its normal oxidized state.

It has been found that in order for hydrogen to be transferred from one compound to another, it is necessary, for some not very well understood reason, that their oxidation-reduction potentials lie rather close together. For example, in the above-mentioned system there would be a much greater difference in oxidation-reduction potential between A and D than between B and C or C and D. Normally one would expect that, with the greater difference of potential, A would transfer hydrogen to D more easily than to C, but this is generally found not to be the case. Hydrogen will be transferred from A to B and B to C and C to D, but A is incapable of transferring hydrogen directly to D at all.

Enzyme inhibitors, or inhibitors of biological action, are usually compounds which destroy or block at least one of a series of catalysts involved in such intermediate reactions, and thus, for practical purposes, prevent the entire reaction. This, for example, is the way many compounds—salts of heavy metals (copper, silver, gold), chlorinated acids, benzoic acid, etc.—used to prevent growth of microorganisms, seem to operate.

In the matter of inhibiting the oxidation of food products, however, the problem becomes somewhat different. Food products are, as a rule, cooked in order to preserve them, and this destroys any enzymes active in their normal biological oxidation processes.

It should be remembered, however, that an enzyme merely slows down or speeds up a reaction and does not change the energetics of a system. That is to say, any reaction which is thermodynamically possible with an enzyme is also thermodynamically possible without the enzyme. The difference is merely one of velocity. Laboratory studies on inhibition of biological oxidations, however, are usually considered in terms of inhibition over a period of minutes or hours, while oxidation of food products may go on and show their effects only after a period of weeks. Thus, it is probably safe to assume that many of the oxidations occurring in food products proceed along the same channels as they would in the raw, uncooked products, the difference being that the enzymes are absent or altered and the velocity much slower.

It would seem, therefore, that a useful antioxidant material might be of two types. It could be of a type that would so change the energetics of the system as to make an oxidation of the food product no longer thermodynamically possible, or it could be one which would not in any way

change the energetics of the system but merely block some one of a chain of reactions, thereby slowing down the oxidation process and increasing the time required for the oxidative defects to occur.

Whether any of the antioxidant materials now in use operate in this latter manner or, if they do, what type of reactions they block, is not very well known. If, however, we assume that in the absence of any enzymes oxidation of an unsaturated, organic molecule proceeds in two steps—first, hydration of the unsaturated molecule, and second, transfer of hydrogen to molecular oxygen to form water—then one type of inhibitor used in biological reactions might possibly indicate a type of compound that would be useful for blocking such oxidations.

In the dissimilation of a substrate medium by *Bacillus coli,* Woolf (1920) has found that there exists an equilibrium between fumaric and malic acids in accordance with the reaction

$$
\begin{array}{c}
\mathrm{COOH} \\
| \\
\mathrm{H-C} \\
| \\
\mathrm{H-C} \\
| \\
\mathrm{COOH}
\end{array}
\quad - \mathrm{H_2O} \rightarrow \quad
\begin{array}{c}
\mathrm{COOH} \\
| \\
\mathrm{CH_2} \\
| \\
\mathrm{HC-OH} \\
| \\
\mathrm{COOH}
\end{array}
$$

malic acid being merely the hydrated form of fumaric acid. He has found, however, that the addition of cyclohexanol completely blocks the hydration of fumaric acid, so that no malic acid at all is formed. This would make it appear that there may be compounds which could block the primary hydration of an unsaturated, substrate molecule and thus prevent any subsequent oxidation, and this may well be the manner in which some antioxidants operate.

That chemical constituents of food materials may require a hydration preliminary to oxidation is evidenced by the fact that alcoholic flavor extracts do not seem to be nearly as susceptible to oxidation as are water solutions or suspensions. Another possible bit of evidence is that flavors seem to deteriorate much more rapidly in acid solution and acids are well known to be active in speeding up hydrolyses in which water is one of the reacting substances.

Carbonated beverages, however, with which this paper is primarily concerned, are sold in hermetically sealed containers and thus the only free oxygen available to bring about oxidation of the product is that amount trapped in the container in the process of manufacture. If, therefore, a compound could be found which is (1) readily oxidized by molecular oxygen, (2) contributes no flavor to the product, either in its reduced or oxidized form, and which (3) in its oxidized form is at such an oxidation potential that it would not subsequently oxidize the ingredients of the beverage it was desired to protect, it would seem that oxidation of carbonated beverages or other products sold in hermetically sealed containers might possibly be prevented. The success of the method would, of course, be dependent upon any reduction of ingredients brought about by excess antioxidant not harmfully altering flavor.

Accordingly a search was made of the literature with a view to finding oxidizable compounds which would possess the aforementioned characteristics and which could possibly serve as antioxidants for food products.

LITERARURE REVIEW

Mattill (1931) found that pyrochatechol, hydroquinone pyrogallol, and hydroxy-hydroquinone served as antioxidants for fats.

Euler, Myrback, and Larsson (1933) isolated vitamin C and tested its oxygen uptake in the Warburg respirometer. They could not get reproducible results in buffered solutions, and assumed it was due to varying amounts of heavy metals which acted as intermediate catalysts between the molecular oxygen and vitamin C. They added KCN to immobilize all the heavy metals and could get no oxidation of the ascorbic acid below pH 9, but above pH 9 the oxidation of the ascorbic acid proceeded rapidly. With metals added as catalysts, they could get appreciable oxygen uptakes by the ascorbic acid at lower pH's. Gluco-reductone was found to have an oxygen uptake practically equivalent to ascorbic acid. Other compounds that proved autoxidizable were dihydroxyacetone and methylglyoxal.

Holtz (1936) found that ascorbic acid markedly accelerated the oxidation of linseed oil. Using a Warburg respirometer he found that the oxygen uptake of linseed oil in the presence of ascorbic acid was 50 to 100 times as great as was the oxygen uptake by linseed oil alone, and was far beyond what could have been accounted for by the oxidation of the ascorbic acid itself. Apparently the linseed oil was at such potential that the ascorbic acid acted as an intermediate catalyst, being alternately oxidized and reduced. However, it exhibited negligible catalytic powers for the oxidation of glucose at pH 7.4.

Guzman, Barron, Demeio, and Klemperer (1936), using ascorbic acid and carefully distilled water, found no oxygen uptake in one hour at pH values up to 7.6, but at this pH the oxidation of the ascorbic acid proceeded rapidly. Copper chloride was found to be an effective catalyst for the autoxidation of ascorbic acid; but manganese, ferrous ammonium sulphate, ferric chloride, cobalt chloride, and calcium chloride would not act as catalysts at pH values between 4.15 and 6.28.

Nakamura and Tomita (1940) found that purified soybean oil was even more unstable to oxidation than was raw oil. By adding the unsaponifiable protein extracted from the oil in a concentration of one per cent the treated oil was made more stable than raw oil.

Maach and Tracy (1939) found oat flour to act as an antioxidant for ice creams and sherbets.

Gray and Stone (1939) found that vitamin C acted as an inhibitor of rancidity and loss of flavor in olive oil emulsions, mayonnaise, salad dressing, dried milk, and eggs.

There have been assigned to the Musher Foundation of New York City 26 patents on antioxidants, many of their antioxidant materials being extracts of cereals, teas, grasses, etc.

From a search of the literature it would seem that such compounds as ascorbic acid, having the endiol grouping COH═COH, or compounds such

as dihydroxyacetone which, judging from their molecular structure, might be capable of giving the endiol grouping, would be the most promising from the standpoint of autoxidizability and prevention of oxidized flavors.

EXPERIMENTAL PROCEDURE

In an attempt to find compounds that might possibly be autoxidizable, that is oxidizable by atmospheric oxygen, in such products as acid beverages, a number of compounds were observed for oxygen uptake in a Warburg-Barcroft respirometer. The compounds employed were ascorbic acid, cysteine monochloride, pyrogallol, pyrochatechol, thiourea, hydroquinone, o-aminophenol, dihydroxyacetone, and the commercial antioxidant preparation from oat flour known as "Avenex Concentrate." All materials were suspended in buffer of pH 5.

Ascorbic acid took up oxygen very rapidly, but the only others that took up any measurable quantities of oxygen at all were pyrogallol and dihydroxyacetone. Accordingly, since we were interested only in compounds autoxidizable at acid reactions, these three were the only ones studied further. Observations were made on the effect of pH of the medium in which they were suspended on the rate of oxygen uptake of these compounds.

The rates of oxygen uptake by ascorbic acid suspended in M/20 phosphate buffers of different pH values were so inconsistent and variable that no conclusions could be drawn as to the effect of reaction on the rate of oxygen consumption of the compound. It was thought that the variability might be due to different amounts of metals in the distilled water, as suggested by Euler *et al.* (1933). Therefore, buffer solutions were made up with a distilled water shown by chemical test to contain less than one part per billion of any heavy metal, but the ascorbic acid still seemed to be oxidized quite rapidly regardless of the pH of the water solution in which it was suspended.

Very consistent results, however, were obtained with the pyrogallol and dihydroxyacetone suspended in solutions of different pH, and pH was found to be a very important factor in the rate at which these compounds were able to take up oxygen.

At pH 7 one ml. of a .1M solution of pyrogallol took up 270 μl. of oxygen in 200 minutes and an equal amount of dihydroxyacetone took up 130 μl. in the same length of time. At pH 5, however, pyrogallol took up but 30 μl. of oxygen in this time and dihydroxyacetone but five μl. t is extremely doubtful, therefore, if either compound would serve to immobilize oxygen in acid carbonated beverages.

This left ascorbic acid as the only compound to be tested. Since it was known that this compound would take up oxygen in acid solutions, further studies were confined to its behavior in orange juice. This medium was chosen because it is the base of many beverages for which antioxidants might be useful.

The oxygen uptake of CO_2 evolution of both pasteurized and unpasteurized juice, each with and without ascorbic acid, were determined. The pasteurization process consisted of holding the juice at a temperature of 60°C.(140°F.) for a period of 10 minutes.

The final mixture in the respirometer cup was 1.5 ml. of orange juice and .5 ml. of either water or a solution of ascorbic acid containing .01 gram per ml. All studies were carried out at a temperature of 31.6°C. (89°F.).

It was found that the juice to which ascorbic acid had been added took up oxygen considerably faster than did those to which ascorbic acid had not been added. It was also consistently found that the pasteurized juice with added ascorbic acid took up oxygen faster than did the unpasteurized, although without added ascorbic acid both juices took up oxygen at approximately the same rate.

A typical result is that in 240 minutes unpasteurized juice, with no added ascorbic acid, took up 35 μl. of O_2 while the pasteurized took up 42 μl. With added ascorbic acid unpasteurized juice took up 67 μl., while the pasteurized took up 110 μl.

Inasmuch as it is often reported in the literature that there is, in fruit juices, a specific enzyme, "ascorbase," which speeds up the oxidation of vitamin C and is inactivated by heat, it is difficult to understand why the pasteurized juice with ascorbic acid should have consistently taken up oxygen faster than did the unpasteurized.

The addition of ascorbic acid did not appreciably affect the rate of CO_2 evolution from either the pasteurized or the unpasteurized juice. However, the rate of CO_2 evolution from the pasteurized juice was always higher than from the unpasteurized. A typical result is that in 240 minutes the unpasteurized juice, both with and without added ascorbic acid, gave off approximately 15 μl. of CO_2 while in the same period of time the pasteurized juice, both with and without added ascorbic acid, gave off approximately 45 μl. of CO_2. This also is difficult to understand.

It was further considered worth while to study the rate at which metals catalytic for oxidations, such as copper and iron, would speed up the oxidation of the antioxidants in orange juice.

This was done by placing in the respirometer 1.5 ml. of pasteurized orange juice, .25 ml. of either a solution of ferrous sulphate containing .64 mg. of ferrous iron or a solution of copper chloride containing 1.0 mg. of copper, and .25 ml. of a M/20 solution of ascorbic acid. The blanks or controls were the same, except that distilled water was substituted for the solution of metal salts.

From the results it is evident that iron is a rather active catalyst for this oxidation and that copper is extremely active (Fig. 1). In the early stages of the oxidation the addition of copper increased the rate of oxidation of the ascorbic acid more than tenfold. It is interesting to consider that both of these elements are metals which show two different valencies and are easily oxidized or reduced from one to the other. It is also interesting that the oxidation-reduction potential of copper +.45 volt (International Critical Tables), and that of iron +.74 volt (International Critical Tables) both lie between that of oxygen +.81 volt, Szent-Gyorgyi (1933), and that of ascorbic acid-dehydroascorbic acid +.112 volt, Oppenheimer and Stern (1939).

These metals were also tested as catalysts for the oxidation of both dihydroxyacetone and pyrogallol suspended in orange juice. Iron increased

the rate of oxidation of each by approximately 50 per cent and copper by approximately 300 per cent. Even so, the rate of oxygen uptake by both pyrogallol and dihydroxyacetone was so slow they were considered impractical for immobilizing oxygen in acid solutions.

EFFECT OF VITAMINS ON FLAVOR OF BEVERAGES

Carbonated beverages seem to be susceptible to two undesirable types of oxidation. One is the oxidative loss of flavor so that the beverage becomes flat and the aroma disappears; the other is the development of oily and bitter tastes, probably due to the oxidation of terpenes borne in the essential oils from which the beverages are made.

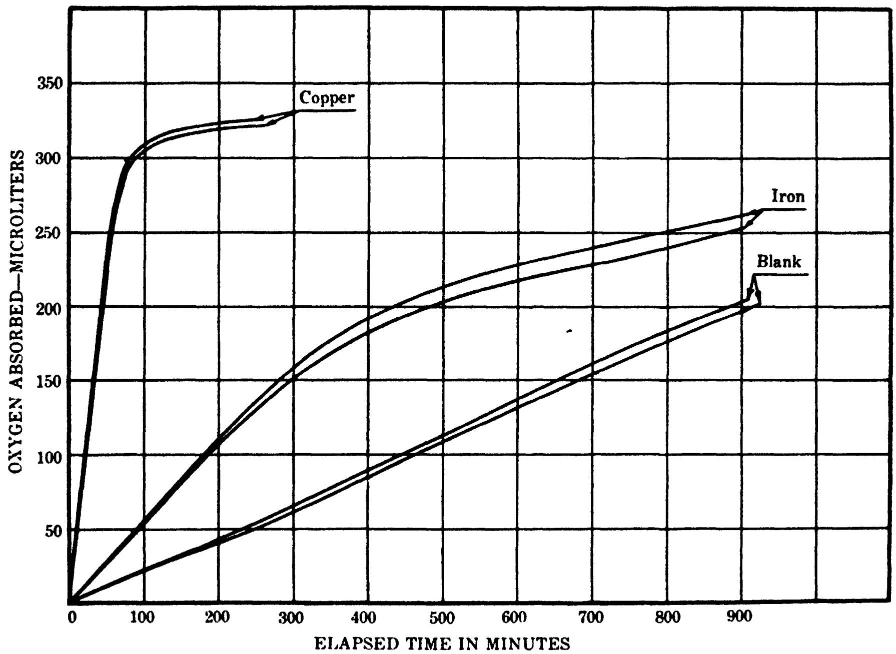

FIG. 1. Effect of catalysts on absorption of oxygen by orange juice containing ascorbic acid.

These terpenes are often removed to some extent, at least, from the essential oils by steam distillation methods or by dilution and absorption, but even so, most true-fruit beverages are still susceptible to the development of the oxidized bitter tastes.

Studies were therefore carried out to determine the power of ascorbic acid to prevent the development of these oxidative defects which usually occur after the beverage has been exposed to strong sunlight. A beverage was made up consisting of three parts of orange juice, one part of 12-per cent sugar solution, and, in order to be sure same terpenes would be present and also to build up the desirable orange flavor, there was added to each six-ounce bottle one-half ml. of an alcoholic, essential-oil solution made by extracting the peeled thin outer skin of four oranges with 150 ml. of 95

per cent alcohol for 25 minutes. The bottles of this beverage were crowned, pasteurized at 70°C.(158°F.) for 10 minutes, cooled, uncrowned, the vitamin C added, and the bottles again resealed.

The amount of vitamin C added was that quantity necessary to take up all the oxygen in the head space of the bottle, assuming 21 per cent oxygen in the air in the head space and all of the oxygen in the liquid, assuming a concentration of eight parts per million of oxygen in the liquid saturated with air. The calculated required amount of ascorbic acid was .08 gram in a six-ounce bottle, but to give a slight excess for a factor of safety, the amount added was one ml. of solution of a gram of ascorbic acid made up to 10 ml., or .1 gram of ascorbic acid per bottle. The ascorbic acid was made up with distilled water and this water, as well as all containers in which it was handled, was sterilized to prevent recontamination of the pasteurized beverages with yeast or mold.

The effect of two types of bottles and exposure to two sources of light on the flavor of beverages were also observed. One type of bottle was a white flint which, according to curves supplied by the Owens-Illinois Glass Company, transmitted all light above approximately 3500Å, while with light of shorter wave lengths percentage transmission decreased rapidly until all light below 3000 Å was cut out. Amber bottles used transmitted approximately 70 per cent of the light of wave lengths above 6200 Å percentage transmission decreasing rapidly with shorter wave lengths until all light below 4400 Å was cut out.

One set of bottles was exposed in a south window, so that any sunlight reaching the beverage passed not only through the bottle but also through the window glass, and another set of bottled beverages was exposed to a commercial 20-watt fluorescent lighting unit, which supposedly radiates light from 3500 Å up.

The antioxidant properties of the ascorbic acid were judged on the basis of both taste and odor developed in the product. All tasting was done at room temperature and odor development was ascertained by sniffing a small sample agitated in a brandy inhalter. It was found that the odor test was much more sensitive than taste and that bad odors could be detected long before any disagreeable taste was found to have developed.

With the use of fluorescent light no bitter taste ever developed in the beverage but there was a very marked loss of flavor. After periods of continuous exposure of as long as two weeks, the control beverages developed a rather smooth, oily taste similar to that of mineral oil. The addition of vitamin C appreciably decreased this loss of flavor. For a considerable length of time the beverage containing vitamin C showed no appreciable change in flavor, and even after such long periods of exposure to fluorescent light as two weeks, they were still much better than the beverages which did not contain vitamin C. In time the beverages developed odors similar to those of decomposed material, but here again, vitamin C markedly inhibited the development of these odors. The use of amber bottles slowed down slightly the oxidative loss of flavor but apparently did not affect the final results since, after a long enough period of time, the taste of the beverages in the amber bottles became just as flat as that of beverages in the ordinary colorless bottles. The period required for the develop-

ment of the loss of flavor was perhaps one-third to one-half again as long in the amber bottles as in the standard colorless glass bottles. Since the amber bottles cut out all wave lengths below 4400 Å, it is suggested that this oxidative loss of flavor is not stimulated exclusively by the ultraviolet rays but that light of longer wave lengths are also effective.

The beverages exposed to the action of sunlight through the window lost flavor and also developed a bitter terpene flavor. The first step in the oxidation seemed to be a loss of flavor, in that the beverage became flat. Later on, the bitter terpene flavor began to appear. A typical set of results on exposure is shown with a beverage made on March 29, 1941. After seven days' exposure, the taste of the beverage containing vitamin C was good and the odor only slightly off, while the taste of the beverage without added vitamin C was slightly off and the odor rather bad. After 20 days the beverage which had received no treatment was very bitter and a beverage through which CO_2 was bubbled just before sealing was also very bitter, while one which contained vitamin C and also had CO_2 bubbled through it prior to sealing still had a good flavor with no evidence of bitterness. The odor of the samples containing vitamin C was very much better than those which did not contain vitamin C.

The use of amber bottles slowed up slightly the loss in flavor and also the development of the bitter tastes but did not in any other way affect the taste finally developed on long standing. It affected only the period of time required to develop the defects, and in about the same degree of magnitude as when exposed to the fluorescent light.

The same concentration of vitamin C, .1 gram per bottle, was also used in a commercial, carbonated, grapefruit-juice beverage. This beverage was packaged in a light green bottle, which would have approximately the same light transmission characteristics as the flint bottle. After a period of exposure of approximately two weeks, the grapefruit beverage containing vitamin C still had, so far as could be detected, its original flavor and almost the original odor, while the same beverage which did not contain vitamin C had lost a great deal of its flavor and had a strong odor of grapefruit peel.

Avenex Concentrate in a concentration of .1 per cent by volume was tested for antioxidant properties against an orange-juice beverage made up in this laboratory but very little evidence of flavor protection was noted.

Since vitamin B_1 is also being incorporated into some beverages it was desired to see what the effect of this vitamin would be on the flavor of the orange-juice beverage made in the laboratory. Using crystalline thiamin chloride, rated at 333 International units per milligram, enough vitamin B_1 was incorporated to give 125 units per six-ounce bottle. The samples were then exposed to the action of sunlight in the window and their flavor compared at various time intervals with those containing no vitamin B_1. It was always found that vitamin B_1 very markedly increased the rate of flavor loss and caused the development of very characteristic and very bad odors. The beverage became flat and tasteless and developed a musty, rubbery odor which could always be identified and was always the same.

A typical case is that of the previously mentioned beverage made up on March 29, 1941, in which, after seven days, the sample to which vitamin B_1 had not been added had a slightly "off" flavor and had a slightly decomposed odor, while in the one containing vitamin B_1 the flavor was rather flat and the odor was very much worse than that of the untreated sample. After 11 days the one without added vitamin B_1 was bitter in flavor, whereas the one containing vitamin B_1 was very flat. After 20 days the one to which vitamin B_1 had not been added was quite bitter, while the one containing vitamin B_1 tasted flat and musty and had a very distinct bad odor. With the addition of vitamin B_1 and C together, the loss of flavor went on much as if no vitamin C had been present. This would tend to indicate that the action stimulated by vitamin B_1 was not simply oxidative in nature or, if so, it is of a type that vitamin C is unable to arrest. Amber bottles slowed down slightly the action of vitamin B_1 but in no other way affected the final results.

It is not entirely surprising to find one vitamin active in preventing flavor loss while another vitamin is very active in producing and stimulating flavor deterioration. In the human body the vitamins, or at least many of them, are thought to be intermediate enzymes active in metabolic processes, and if a vitamin be an oxidation-reduction catalyst, whether it will prevent flavor deterioration or stimulate it will depend upon the potentials of the hydrogen donators and hydrogen acceptors active in the system. Thus, while ascorbic acid acts as a flavor protector for some types of fat emulsions and, as we have found, for beverages, Holtz (1936) found with a different type of material, namely linseed oil, that vitamin C acted as a very strong catalyst for its oxidation.

Since vitamin B_1 in its active phosphorylated form, co-carboxylase, is thought to be active in decarboxylation reactions, the respiration of both pasteurized and unpasteurized orange juice containing both vitamin B_1 and co-carboxylase, each with and without added magnesuim, were studied. As far as could be determined with the Warburg-Barcroft respirometer no stimulation of either CO_2 evolution or oxygen uptake by any combination could be found.

Since the beverages containing vitamin B_1 and exposed to sunlight seemed to be extremely flat and lacking in acid taste, it was thought that the vitamin B_1 might be bringing about a decarboxylation of the citric acid of the beverage and thereby a decrease in the acid content. Titration, however, failed to reveal any decrease in acid content of the beverages to which vitamin B_1 had been added.

SUMMARY

The rate of absorption of oxygen by orange juice in the presence of ascorbic acid was increased by pasteurization. In the absence of added ascorbic acid oxygen uptake was 35 μl. for the unpasteurized and 42 μl. for the pasteurized juice, whereas in the presence of added ascorbic acid the respective oxygen uptakes under the same conditions were 67 and 110 μl.

Presence of iron, and particularly copper, very markedly increased the rate of absorption of oxygen by orange juice to which ascorbic acid had been added.

The addition of Avenex Concentrate did not appreciably affect the period of flavor retention.

Incorporation of vitamin B_1 in an orange beverage distinctly accelerated the rate of loss of flavor and resulted in the development of bad taste and a characteristic musty and rubbery odor.

Addition of ascorbic acid to true-fruit orange and grapefruit beverages in concentrations in excess of that required to take up the free oxygen in the container, distinctly increased the period of retention of the normal taste and flavor.

Whether or not the addition of ascorbic acid increases the keeping qualities of beverages sufficiently to make the practice commercially feasible, would probably be determined primarily on economic grounds. The results obtained indicated clearly the importance of keeping oxygen away from beverages containing fruit juice, in all stages of their manufacture.

ACKNOWLEDGMENT

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EFFECT OF ACIDS AND SUGAR ON VIABILITY OF *ESCHERICHIA COLI* AND *EBERTHELLA TYPHOSA*[1]

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The general objective of the studies reported herein was to learn more about the behavior of intestinal organisms in acid solutions simulating carbonated soft drinks, in order to determine the possibilities of transmission of water-borne diseases by such beverages. The specific objectives of the studies were to obtain information as to the following:

(1) The order of effectiveness, against intestinal organisms, of the various edible acids at equal normality and in a concentration approximating that found in carbonated beverages.

(2) Relative rates of death of *Escherichia coli* in acid solutions and in water at ordinary summer temperature of approximately 30°C.(86°F.) and beverage-cooler temperature of approximately .6°C.(33°F.).

(3) Effect of CO_2 and sucrose on survival of *Escherichia coli* exposed to lactic and citric acids.

(4) Comparison of the resistance of *Esch. coli* and *Eberthella typhosa* to acid solutions.

(5) Survival of *Esch. coli* in commercial beverages in relation to sugar, acid, and gas content.

(6) The effect of size of inoculum on the death rate of *Esch. coli* in acid solutions.

(7) Probable time that beverages, made up with the different acids studied, would have to be stored in order to reduce accidental infections of various magnitudes to a point where the beverages would meet the U. S. Public Health Service standard for drinking water.

REVIEW OF LITERATURE

Paus (1908) studied the inhibition, by acids, of growth of colon and typhoid organisms in bouillon, employing 20 organic and three inorganic acids, and in every case more acid was required to prevent the growth of coliform bacteria than the typhoid bacillus.

Cohen (1922) calculated the ratio of K values for the typhoid to those for coliform organisms in Clark and Lub's phthalate-phosphate buffers at definite hydrogen-ion concentrations for temperatures ranging from 0 to 30°C.(32 to 86°F.). The ratios increased progressively from 8 at 30°C. to 67 at 0°C., indicating that the typhoid bacillus died much more rapidly than did *Esch. coli* and that this was particularly true at the lower temperature.

[1] These studies were made possible through a fellowship maintained by the American Bottlers of Carbonated Beverages at Iowa State College.

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Reid (1932) reported that 50 to 100 per cent more of a number of inorganic acids was necessary to kill the colon organisms in 15 minutes than was required to kill the typhoid organism.

Koser and Skinner (1922) found that the typhoid organism died faster than did *Esch. coli* in tap water contianing 3.5 volumes of CO_2.

Kitasato (1888) determined the amount of various acids required to kill typhoid organisms in bouillon in three to four hours. On a weight basis, the order of effectiveness of the acids studied was acetic > lactic > phosphoric > oxalic > citric > tartaric = malic. Recalculating his results on a normality basis does not change the order appreciably, but the effectiveness of the acetic and lactic acids is brought very close together.

Paus (1908) found that for preventing growth of coliform and typhoid bacilli in bouillon the order of effectiveness of a number of acids, on a weight basis, was acetic > phosphoric > tartaric > citric > lactic. When his results are recalculated on a normality basis, however, the order becomes tartaric = lactic = acetic > citric > phosphoric.

Wyeth (1918) reported that in a .2-per cent peptone medium the order of effectiveness against growth of coliform bacteria on a pH basis was acetic > lactic > HCl.

Reid (1932) found that on a normality basis the order of effectiveness for killing *Bacterium pyocyaneus* in 15 minutes at 20°C. (68°F.) was oxalic > glycolic > lactic > citric > propionic > acetic.

Fabian and Wadsworth (1939) reported that, on a basis of equal titratable acidity, acetic acid was greatly superior to lactic acid for killing an aciduric yeast in canned pickles, even though the pH of the lactic acid solution was much lower.

Nunheimer and Fabian (1940) studied the germicidal and inhibiting effects of various acids on staphylococci in dextrose broth. On a pH basis the order of effectiveness for germicidal action of the acids studied was acetic > citric > lactic > malic > tartaric > HCl, while the order for growth-inhibiting properties was the same except that citric and lactic acids changed places in the series. On the basis of equal titratable acidity, however, the order would be HCl > lactic > tartaric > acetic > malic > citric, as respects germicidal powers, while for growth inhibition HCl and lactic acid would change places.

Levine and Fellers (1940) observed that acetic acid was always much more effective than either HCl or lactic acid against growth of the yeast *Saccharomyces cerevisiae* in 48 hours or for killing *Salmonella aertrycke* in 15 minutes.

Leone (1885) found that, whereas the number of organisms increased markedly in uncarbonated Munich tap water, there was observed a regular and continual decrease in carbonated tap water. The organisms also increased in tap water through which hydrogen had been bubbled so he concluded that a specific germicidal effect was exerted by CO_2 and that the decrease was not due merely to lack of oxygen.

Hoffman (1906) reported that in order to sterilize raw river water in relatively short periods of time with carbon dioxide, it was necessary to use pressures of approximately 50 atmospheres and that at this pressure

water suspensions of typhoid, cholera, and dysentery bacilli were killed in three hours.

Whipple and Mayer (1906) observed that typhoid organisms died faster in distilled water kept under an atmosphere of CO_2 than in distilled water kept under air, while Young and Sherwood (1911) found that intestinal organisms died faster in carbonated samples of "bottler's lemon" than in uncarbonated samples.

Gershenfeld (1920) noted that the bacterial count of a carbonated beverage was lower than the water from which it was made.

Koser and Skinner (1922) reported that *Bacillus coli* died faster in carbonated than in uncarbonated city tap water, and also that it died more quickly in an acid lemon soda than in a non-acid vanilla soda.

Donald, Jones, and MacLean (1924), using an "ordinary ginger ale" inoculated with colon and typhoid bacilli, found that the numbers in uncarbonated samples rose while the numbers in carbonated samples decreased steadily. Apparently their ginger ale contained no acid, for such organisms could not multiply in a beverage as acid as ginger ale ordinarily is.

Guillerd and Lieffrig (1935) inoculated tap water saturated with carbon dioxide with colon and typhoid organisms and obtained no reduction in numbers of *B. coli* in 60 minutes and no reduction in typhoid organisms in 30 minutes.

Swearingen and Lewis (1933) found that CO_2 pressures of 350 to 400 pounds per square inch over a water suspension of coliform bacteria killed 95 to 99 per cent of the exposed organisms in 20 minutes.

EXPERIMENTAL PROCEDURE

The test organisms used in this study were the Hopkins strain of *Eberthella typhosa* and a typical *Escherichia coli*, culture No. 601 of the Iowa State College collection. The inocula were prepared by appropriate dilution of a 24-hour broth culture of the organism which had been centrifuged and resuspended in cold, sterile tap water. The purpose of centrifuging and resuspending was to avoid carrying over any appreciable amount of nutrients with the inocula.

It was originally intended to make up the acid solution with a synthetic tap water prepared by adding salts to laboratory distilled water. However, the distilled water itself was found to be so germicidal, probably owing to presence of heavy metals, that this attempt had to be abandoned and the acid solutions made from college tap water which had been boiled and filtered in order to remove the precipitated temporary hardness, thus giving a water of practically constant composition that was not itself germicidal and which did not give rise to any precipitate upon further sterilization.

The acid solutions were prepared by adding to the above-mentioned water enough of a two normal solution of the desired acid to the water to give a final concentration of .02 N or approximately 70 grains per gallon calculated as citric acid. These acid solutions were then dispensed into regular six-ounce beverage bottles, crowned, and sterilized in the autoclave. The hermetically sealed container prevented the loss of any acid during the process of sterilization.

Samples to be carbonated were cooled to .6° C. (33° F.), opened, a weighed amount of dry ice dropped into the bottle, the bottles crowned quickly and shaken in an international shaking machine while the dry ic dissolved in the liquid.

To study the rate of death of an organism in a given solution, bottles containing this solution were brought to the temperature at which they were to be incubated, then inoculated, crowned, mixed, and stored at the desired temperature. Samples to be stored at 30°C. (86°F.) were kept in the laboratory incubator, while those to be stored at .6°C. were kept in a large cold-storage room maintained at this temperature.

At appropriate time intervals a bottle was removed from the incubator, or cold-storage room, and the number of surviving organisms ascertained. Determination of numbers of *Esch. coli* surviving were made by plating onto Bacto-Endo agar, if over 14 per ml., and all numbers from 14 to .004 per ml. were determined by placing three 10-ml., three 1-ml. and three .1-ml. portions into lactose broth, then obtaining the M.P.N. from the McGrady (1918) tables. The lactose broth consisted of two per cent Bacto-tryptose, .5 per cent lactose, .5 per cent dipotassium-phosphate, and .5 per cent sodium chloride made up with distilled water.

Incidence of organisms other than colon forms was found to be so rare as not to affect the results and therefore no attempt was made to use differential media for determining numbers of surviving typhoid organisms. Plate counts of surviving typhoid bacilli were made on nutrient agar containing .2 per cent glucose for counts over 14 per ml., and lactose broth (multiple tube dilution) was used for counts of 14 to .004 per ml. as previously described.

RESULTS AND DISCUSSION

The results presented in the graphs are averages obtained in the following manner: The data for two or three runs were plotted and averaged by reading from the individual plots the times required to effect specified percentage reductions in numbers. These values were then plotted to obtain the average survivor curves shown (Figs. 1 to 8).

Relative Germicidal Efficiency of .02 N Edible Acids at 30°C.: Survivor curves for *Esch. coli* suspended in water and in .02 N acids at 30°C. (Fig. 1) show that *Esch. coli* died considerably faster in all of the acid solutions than in water and that the order of effectiveness of the acids was tartaric > glycolic > phosphoric > lactic > acetic > citric. This order is at variance with the recalculated results of Kitasato (1888) chiefly in the position which tartaric acid takes in the series, and from those of Paus (1908) for inhibition of growth of colon and typhoid forms, chiefly in the position which phosphoric acid takes in the series. The results are in agreement with those of Reid (1932) for killing *B. pyocyaneus* in 15 minutes, except that he found acetic acid to be less effective than citric.

The order of effectiveness of the acids reported herein is the same as that shown in the results of Nunheimer and Fabian (1940) for killing and inhibiting growth of staphylococci and with the recalculated results of Levine and Fellers (1940) for killing Salmonella in 15 minutes.

It should be borne in mind that the results obtained for killing organisms in periods as short as 15 minutes are of limited practical application,

since the concentration of acids required for such a germicidal effect are generally so high as to render any material containing them unfit for human consumption.

The results of Levine and Fellers (1940) and of Fabian and Wadsworth (1939) show acetic acid (on an equal normality basis) is more effective against yeast than is lactic acid, but the results reported herein, as well as those found in the literature, would indicate that the reverse is true for bacteria.

It is interesting to examine the effectiveness of these acids from the viewpoint of their chemical structure. Glycolic acid, which is acetic acid with a hydroxyl substituted for one of the hydrogens of the methyl group, is almost four times as effective, from the standpoint of time required to effect a 99.99-per cent reduction in numbers, as is acetic acid. Lactic acid,

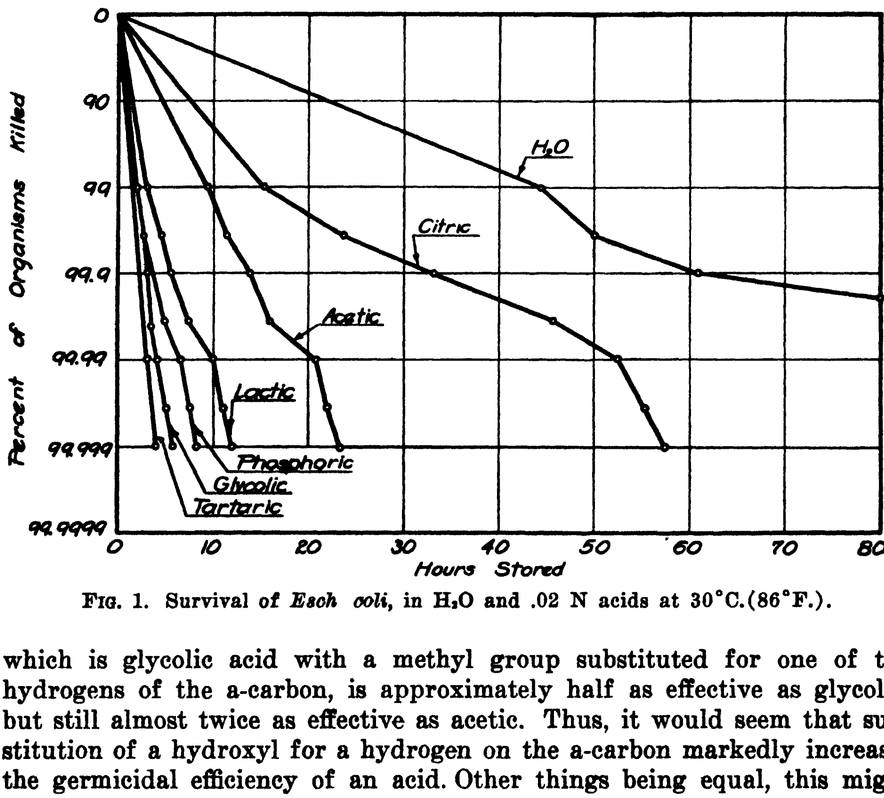

FIG. 1. Survival of *Esch coli*, in H_2O and .02 N acids at 30°C.(86°F.).

which is glycolic acid with a methyl group substituted for one of the hydrogens of the a-carbon, is approximately half as effective as glycolic, but still almost twice as effective as acetic. Thus, it would seem that substitution of a hydroxyl for a hydrogen on the a-carbon markedly increases the germicidal efficiency of an acid. Other things being equal, this might be expected from the general rule that the substitution of a negative group for a positive group on the alpha carbon increases the acidity of the carboxyl. This is further borne out by the fact that tartaric acid, which is very effective against *Esch. coli,* has hydroxyl groups on each of the carbons attached to its carboxyl group and might be considered as two molecules of glycolic acid hooked together.

Citric acid, however, has a hydroxyl group on the carbon next to only one of its three carboxyl groups, leaving two of its carboxyls without this apparently effective grouping. It might at first seem that the lesser

germicidal efficiency of citric acid could be explained by the fact that it is a bulky molecule and might penetrate the cell slowly, but tartaric acid should also be a rather bulky molecule and, judging from its effectiveness, this acid apparently penetrates quite well.

Another possible explanation of the slow action of citric acid would be the fact that it is tribasic, and therefore on an equal normality basis there would be only one-third as many molecules present as there would be with a monobasic acid. The high effectiveness of tartaric acid, however, tends to nullify this possible argument since tartaric acid is dibasic and there would be only half as many molecules present as there would be with glycolic acid, yet tartaric acid is somewhat more effective on a normality basis than glycolic.

Effect of Temperature on Rate of Death of Esch. coli in .02 N Edible Acids: Survivor curves for *Esch. coli* in .02 N solutions of citric, lactic,

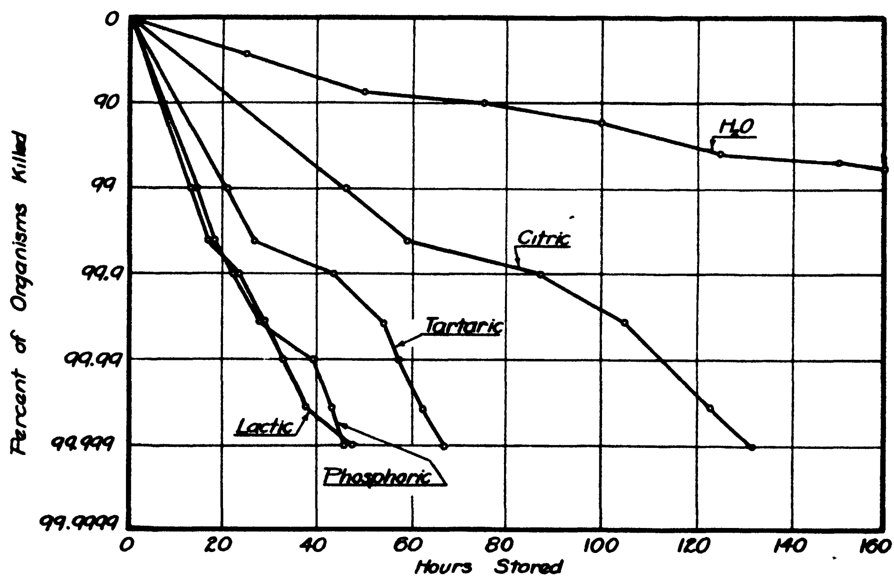

FIG. 2. Survival of *Esch. coli* in H_2O and .02 N edible acids at .6°C.(33°F.).

phosphoric, and tartaric acids in water at .6°C. (Fig. 2) indicate that the germicidal activities of lactic and phosphoric acids are highest and very close together, that tartaric acid is less effective than either of the foregoing, and that citric acid is the least effective germicide. The ratio of the rates of death at 30°C. to the rates of death at .6°C. for the acids studied were as follows: citric 2:3; lactic 3:9; phosphoric 5:4; and tartaric 15:8. Thus it appears that, as would be expected, the death rates of the organisms in acid solutions were very much slower at the lower temperatures but that, quantitatively, the effect of temperature is different with different acids. Whereas at 30°C. the order of germicidal efficiency was tartaric > phosphoric > lactic > citric, at the lower temperature, .6°C., the order becomes lactic = phosphoric > tartaric > citric. Tartaric acid seems particularly affected by change of temperature.

Effect of Carbon Dioxide and Sucrose on Survival of Esch. coli in .02 N Citric and Lactic Acids: A strain of *Esch. coli* suspended in boiled tap water containing 2.5 volumes of carbon dioxide died off slowly at about the same rate as in uncarbonated water, a reduction of 99.99 per cent being effected in 155 hours when stored at 30°C. Reports in the literature indicate that coliform bacteria die off more rapidly in carbonated water, and observations in our laboratories have confirmed this with many strains, but from time to time, strains have been encountered which were not appreciably affected by CO_2 in concentrations of 2.5 to 3.0 volumes.

The effect of the presence of sucrose and carbon dioxide on survival of *Esch. coli* in .02 N citric and lactic acids, respectively, is shown (Figs. 3 and 4). Carbon dioxide alone was only slightly germicidal and the organisms grew slowly in the 10-per cent sucrose solution, but it is apparent that addition of sucrose or CO_2 in presence of citric or lactic acid increases appreciably the rate of death of *Esch. coli.* The effects, however, were of different degree for the two acids employed.

Considering lactic acid, it will be noted that killing time of 12.2 hours by .02 N acid alone, was reduced to 9.7 hours by 2.5 volumes of CO_2 (a reduction of 21 per cent) and to nine hours (a reduction of 26 per cent) when 10 per cent sucrose was present, the effects of the concentration of CO_2 and sucrose employed being therefore about the same.

The results with citric acid were particularly marked and striking. The killing time for .02 N citric acid alone was 57.5 hours. The presence of 10 per cent sucrose reduced the killing time by 44 per cent to 32 hours, and incorporation of 2.5 volumes of CO_2 resulted in decreasing the killing time by 71 per cent to 17.5 hours. The increased germicidal efficiency induced by the presence of CO_2 is particularly noteworthy for the results cannot be explained by a simple addition effect of CO_2 and citric acid, but rather indicate an associative action which probably affects the permeability of the bacterial cell.

Relative Resistance of Esch. coli and E. typhosa to Carbon Dioxide and Lactic and Citric Acids: Survivor curves for *Esch. coli* and *E. typhosa* exposed to a pressure of 42 pounds (2.5 volumes) carbon dioxide and to .02 N lactic and citric acids at a temperature of 30°C. (Fig. 5) show that with each of the acids the typhoid organism died much more rapidly than did *Esch. coli.* Thus in CO_2 the time to effect a reduction of 90 per cent of *Esch. coli* was 28 hours as compared with only six hours for *E. typhosa*; 99.99 per cent of the latter were killed on exposure for about 30 hours, if the initial inoculum was 350 per ml., indicating that *E. typhosa* is quite readily killed by 2.5 volumes of CO_2 at 30° C.

Citric and lactic acids are much more germicidal than CO_2, as has previously been pointed out, and they are considerably more effective against *E. typhosa* than towards *Esch. coli.* Thus with .02 N citric acid 32 hours were required to effect a reduction of 99.9 per cent *Esch. coli* and less than 10 hours for *E. typhosa.* With lactic acid (.02 N) there was obtained a reduction of 99.9 per cent *Esch. coli* in approximately six hours as compared with less than one hour for *E. typhosa,* and the times to effect reduction of 99.99 per cent were nine hours and about one hour for *Esch. coli* and *E. typhosa,* respectively. The ratios of death times of *E. typhosa*

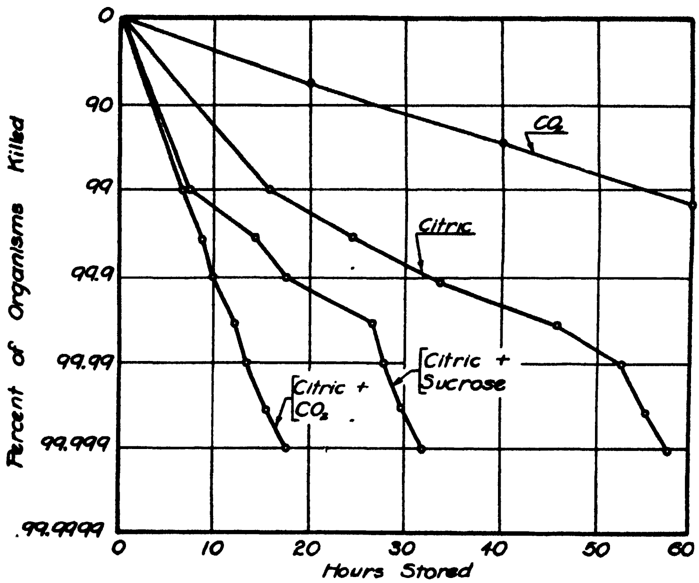

FIG. 3. Effect of 10 per cent sucrose and CO_2 (42 lb./in.^{2} or 2.5 vols.) on the survival of *Esch. coli* in .02 N citric acid at 30°C.(86°F.).

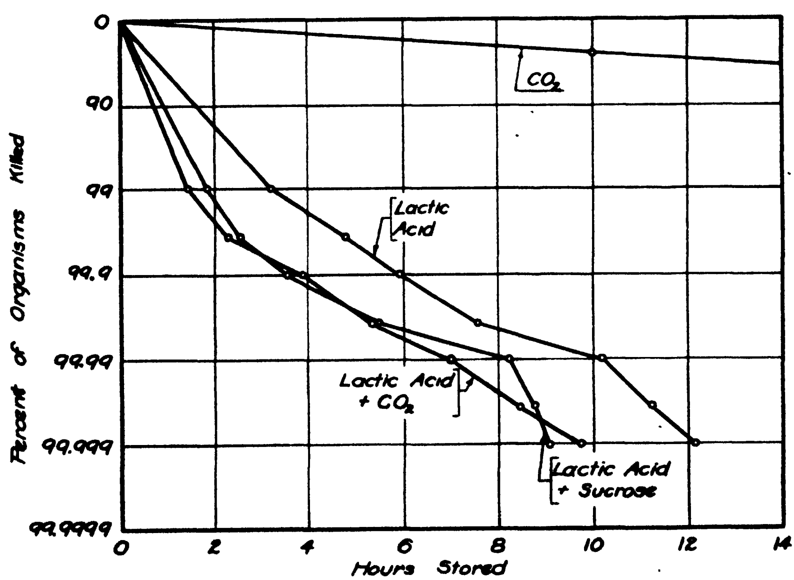

FIG. 4. Effect of 10 per cent sucrose and CO_2 (42 lb./in.^{2} or 2.5 vols.) on the survival of *Esch. coli* in .02 N lactic acid at 30°C.(86°F.).

to those of *Esch. coli* were about five, four, and eight in CO_2, citric acid, and lactic acid, respectively.

The foregoing results are in line with those of Paus (1908), Reid (1932), Young and Sherwood (1911), Koser and Skinner (1922), and Donald, Jones, and MacLean (1924), all of whom found the colon organism to be more resistant to CO_2 and the acids with which they worked than was the typhoid organism.

It would seem, therefore, that there is little doubt that *Esch. coli* is more resistant to acid solution and to CO_2 than is *E. typhosa*, and that the colon organism would satisfactorily serve as a sanitary index for acid and carbonated beverages.

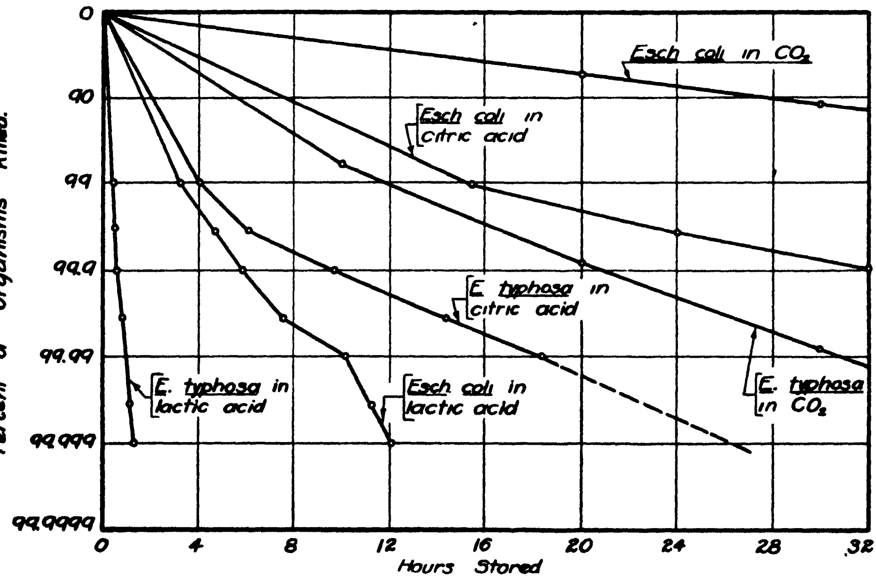

FIG. 5. Survival of *Esch. coli* and *E. typhosa* in .02 N citric acid and lactic acid and 42 lb./in.^{2} (2.5 vols.) CO_2 at 30°C.(86°F.).

Survival of Esch. coli in Commercial Soft Drinks: The death rates of *Esch. coli* in carbonated beverages containing citric or phosphoric acids employing a storage temperature of .6°C. were determined. While the variation in composition of the beverages does not allow direct comparison to be made with the data obtained for pure acid solutions, the results indicated that the death rates were in line with those to be expected on the basis of the acid, sugar, and CO_2 in the beverage.

The time required to effect a reduction of 99.999 per cent of *Esch. coli* in a beverage (ginger ale) containing .017 N citric acid and four volumes of CO_2 was 245 hours, while with a pure solution of .02 N citric acid, without sugar or CO_2, the killing time was 140 hours. Thus, the killing time for the ginger ale was about twice as long as what would have been expected for the acid alone. In a beverage containing .026 N phosphoric acid and 3.2 volumes of CO_2 the killing time was 25 to 30 hours, while .02 N phosphoric acid alone showed a killing time of 45 hours. The observation with

the latter beverage is in line with what was observed with a mixture of acid, sugar, and CO_2.

Effect of Size of Inoculation on Death Rate of Esch. coli in Acid Solution: It was desired to ascertain the range of organism population through which the constant death rate, observed in the acid solutions during the course of this study, would hold and also to determine whether the size of the inoculum had any effect on the death rate. The results for three widely different inocula in .02 N lactic acid are shown (Fig. 6). It will be noted that the semilogarithmic death plots for inocula of 45, 4.500, and 450,000 organisms per milliliter are all essentially straight lines which are also parallel.

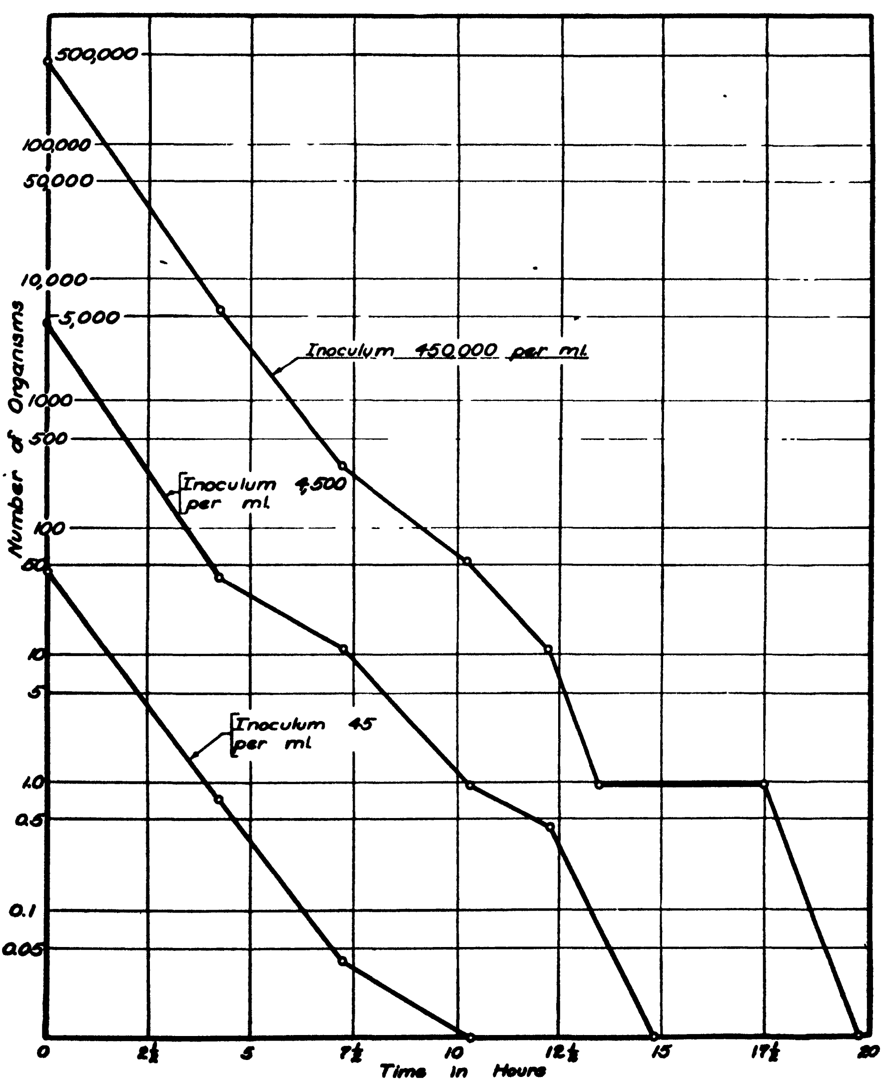

FIG. 6. Effect of size of inoculum on death rate of *Esch. coli* in .02 N lactic acid at 30°C.(86°F.).

The results of this experiment seem either to refute the often proposed argument that in a given sample of organisms there is a certain proportion of highly resistant forms which will remain alive or to indicate that such resistant forms are very few in number.

Effect of Initial Inoculum on Time Required to Meet Drinking Water Standards: Assuming, as would seem justifiable, a constant death rate (semilogarithmic) for *Esch. coli* in solutions of the various acids used in this investigation, there have been computed and recorded the probable lengths of time it would be necessary to store beverages made from these acids, at different temperatures and with varying degrees of initial contam-

TABLE 1

Coefficients of Rates of Death (K Values) for Escherichia coli in .02 N Solutions of Various Acids

Acid	K value
Temperature 30°C. (86°F.)	
1. Citric in H_2O	0.862×10^{-1}
2. Acetic in H_2O	2.13×10^{-1}
3. Lactic in H_2O	4.35×10^{-1}
4. Phosphoric in H_2O	6.67×10^{-1}
5. Glycolic in H_2O	9.1×10^{-1}
6. Tartaric in H_2O	12.5×10^{-1}
7. Citric in 10% sucrose	1.56×10^{-1}
8. Citric + 3-vol. CO_2	2.86×10^{-1}
9. Lactic in 10% sucrose	5.56×10^{-1}
10. Lactic + 3 vol. CO_2	5.56×10^{-1}
Temperature .6°C. (33°F.)	
1. Citric in H_2O	0.37×10^{-1}
2. Tartaric in H_2O	0.75×10^{-1}
3. Phosphoric in H_2O	1.16×10^{-1}
4. Lactic in H_2O	1.16×10^{-1}

ination, in order that they meet U. S. Treasury Department Bacteriological Standards for drinking water. "K" values for the various solutions are listed (Table 1) and were computed from the formula $K = \frac{\log B - \log b}{t_2 - t_1}$; "t" was expressed as "time in hours," "B" and "b" being the numbers of surviving organisms at times "t_1" and "t_2," respectively. When this basic formula is transposed to read in percentage of organisms living rather than numbers of surviving organisms, it turns out that K = five (5) divided by the time in hours required to effect a 99.99 per cent reduction in numbers. The "K" values listed in Table 1 were computed by dividing 5 by the time in hours required for a given solution to effect this reduction in numbers.

The times required for acid solutions at 30 and .6°C. to reduce various initial inocula to a point where the U. S. Treasury Department standard for drinking water would be met have been computed and are listed

(Table 2). It will be noted that when the inoculum was increased 100-fold the time required was doubled.

TABLE 2

Hours Required for .02 N Acid Solutions Containing Various Numbers of Escherichia coli to Meet U.S.P.H.S. Standard for Drinking Water[1]

Initial colon index[2]	10,000	5,000	1,000	500	100
	Stored at 30°C. (86°F.)				
Citric in H_2O	46.5	42.9	34.8	31.2	23.2
Acetic in H_2O	18.7	17.3	14.09	12.6	9.3
Lactic in H_2O	9.2	8.5	6.8	6.2	4.6
Phosphoric in H_2O	6.0	5.5	4.5	4.06	3.0
Glycolic in H_2O	4.4	4.07	3.3	2.9	2.2
Tartaric in H_2O	3.2	2.9	2.4	2.1	1.6
Citric in 10% sucrose	25.6	23.6	19.2	17.3	12.8
Citric + 3 vol. CO_2	13.9	12.9	10.5	9.4	7.0
Lactic in 10% sucrose	7.2	6.6	5.3	4.7	3.5
Lactic + 3 vol. CO_2	7.2	6.6	5.3	4.7	3.5
	Stored at .6°C. (33°F.)				
Citric in H_2O	108.0	100.0	81.2	73.0	54.1
Tartaric in H_2O	53.4	49.4	40.0	36.0	21.6
Phosphoric in H_2O	34.5	31.9	25.8	23.2	17.2
Lactic in H_2O	34.5	31.9	25.8	23.2	17.2

[1] One (1) per 100 ml. [2] Number of *Escherichia coli* per 100 ml.—initial inoculum.

SUMMARY

In a concentration of .02 N, which is approximately that employed in many beverages, the order of effectiveness of edible acids as germicides against *Esch. coli* at 30°C. was tartaric > glycolic > phosphoric > lactic > acetic > citric.

The temperature coefficients of the rates of death are quite different for the various edible acids studied. Thus, considering the four acids observed at 30°C. (86°F.) and .6°C. (33°F.), the ratios of the killing times at the higher to that at the lower temperature were as follows: citric acid 2:3; lactic acid 3:9; phosphoric acid 5:4; and tartaric acid 15:8. Consequently, the order of effectiveness was different at the two temperatures—the order tartaric > phosphoric > lactic > citric at 30°C. becoming phosphoric = lactic > tartaric > citric at .6°C.

Comparing the germicidal efficiencies of acetic, lactic, and glycolic acids, it appears that the substitution of an hydroxal (OH) for one of the hydrogens on the alpha carbon atom markedly increases the germicidal efficiency of an acid.

The germicidal efficiency of .02 N lactic acid at 30°C. was increased about to 20 to 25 per cent by the addition of 2.5 volumes carbon dioxide or 10 per cent of sucrose.

A concentration of .02 N citric acid at 30°C. required 57.5 hours to kill 99.999 per cent of exposed cells of *Esch. coli* at 30°C. Addition of 10 per cent sucrose reduced the killing time by about 40 per cent to 32 hours. Incorporation of 2.5 volumes of carbon dioxide with the citric acid, reduced the killing time by about 70 per cent to 17.5 hours.

The increased germicidal action induced by CO_2 cannot be explained as a simple additive effect of CO_2 and citric acid, but rather indicates an associative action which probably affects permeability of the bacterial cell.

E. typhosa was much more susceptible to the effect of CO_2 and edible acids than was *Esch. coli*, the time to effect a stipulated reduction being about five times as long for *Esch. coli* as for *E. typhosa*, with 2.5 volumes of CO_2, four times as great for .02 N citric acid, and eight times as long in the presence of .02 N lactic acid.

The rate of death of *Esch. coli* at 30°C. was constant throughout the course of disinfection with .02 N lactic acid and independent of the size of inoculum.

A table is presented indicating the storage periods required to reduce various inocula of *Esch. coli* in .02 N edible acids with or without CO_2 or sugar to a point of compliance with the United States Public Health Service standards for drinking water.

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EFFECT OF FILTRATION ON APPEARANCE, VISCOSITY, AND ALCOHOL-INSOLUBLE FRACTIONS OF APPLE JUICE[1,2]

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The commercial processing of apple juice has received considerable investigation during the past decade. Carpenter and Walsh (1932), Pederson and Tressler (1938), Mottern, Neubert, and Eddy (1940), and others have shown that when suitable methods of pasteurization are used apple juice may be preserved without imparting a "scorched" or "cooked" flavor to the juice. Methods of clarification have been improved to the point where "off" flavors are not imparted to the juice, and specially lacquered tin containers have been developed which will retain juice for extended periods without deterioration. In spite of these developments preserved apple juice does not enjoy the public acceptance which might be expected from its popularity in the fresh state or from the high popularity of processed fruit juices in general.

Because processed apple juice is considered by many as inferior to the freshly pressed juice or apple cider a study was undertaken to determine some of the changes which occur in juice during the processing operations. Practically all apple juice, with the exception of the recently developed pulpy juice or apple crush, is subjected to a filtration process. This filtration may vary in degree from straining through cloth, to remove large apple pieces, to more thorough filtrations which yield brilliantly clear juice.

This investigation was devoted to a study of changes in appearance, viscosity, and alcohol-precipitate and pectic-acid fractions, which occur in apple juice as a result of the filtration process. Seven grades of Seitz filter sheets and Whatman No. 2 filter paper were used as filters. Changes caused in juice owing to filtration were compared with those caused by centrifuging juice. The study includes the filtration of unclarified as well as clarified juices.

LITERATURE

Information available on changes in apple juice as a result of filtration is limited. Filtration of apple juice through a Seitz sterilizing filter adversely affected the color and the "body" of apple juice, according to Charley (1932). He stated that the removal of color by Seitz EK filters was probably due to adsorption of colloidal tannin complexes by the film.

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[2] From a portion of a thesis submitted by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Chemistry, State College of Washington, 1941.

Charley (1933) stated that pectin gave "body" to apple juice but made filtration difficult and that centrifuged juice had more flavor than filtered juice but was less clear. According to investigations by Charley and Harrison (1939), juice which had been pasteurized was fuller in total flavor, heavier in "texture," and possessed a more mellow and less acid character than juice which had been sterile-filtered. Germ-proof filtration was found by Clague and Fellers (1936) to remove much of the color and "body" from cider.

Carpenter and Walsh (1932) reported that apple juice which was flash-heated and then filtered through a Seitz sterile filter lost from 25 to 51 per cent of its nitrogen content, as much as 83 per cent of the tannin substances, no non-tannin astringency, and from 24 to 49 per cent of the pectin substances. The loss in tannin and nitrogen was attributed to the filtration step in the treatment, whereas the heat treatment was considered responsible for the loss in pectin substances.

The viscosity or "body" of apple juice varies with the variety of fruit used and with its maturity. Clague and Fellers (1936) found that cider became more viscous as the fruit became more mature. This is in agreement with work of Carre (1925) in which it was shown that during storage the soluble pectin content of apples increased at the expense of insoluble pectose.

Grove (1930, 1931) reported that juice made from apple varieties grown in Nova Scotia and British Columbia lacked "body" in comparison with that made from English cider apples. He associated "body" with tannin content rather than pectic substances. From these reports it is apparent that flavor is not the sole criterion in the quality of apple juice, as the colloidal pectic substances associated with "body" do not contribute directly to the flavor.

ANALYTICAL METHODS

Alcohol precipitate and pectic acid were determined by the methods described on pages 324 and 325 of the *Official and Tentative Methods of Analysis*, Association of Official Agricultural Chemists, fourth edition. Viscosity was measured at 20°C.(68°F.) by means of an Ostwald pipette held in a constant temperature bath. Results are expressed in centipoise units using water for standardization. Total astringency was determined by adapting the method of Lowenthal-Proctor (Griffin, *Technical Methods of Analysis*, pages 95-96 and 479-480) to apple juice. Ten milliliters of Indigo Carmine solution and 25 ml. of apple juice were titrated with N/20 $KMnO_4$ solution. This method gave a blank titration of six ml. and a total astringency titration varying from 12 to 18 ml. Results are expressed in terms of tannin. Ash determinations were made on the filter sheets by heating the sample to red heat in a platinum crucible over a Bunsen burner.

Juice samples were examined for relative clarity by both transmitted and reflected light. In determining the clarity by transmitted light the samples were held between the light source and observer. For clarity by reflected light the samples were placed over a small circular opening behind which the light source was placed and the clarity noted at a 90-degree angle to the light source.

PREPARATION OF SAMPLES

Five lots of apple juice, designated as F1, F2, F3, F4, and F5 (Table 2), were prepared for this study. Lot F1, used in studying the Seitz series of filter sheets, was prepared from whole, sound Winesap apples by grinding in a grater-type mill and pressing with a hydraulic press using racks and cloths. All samples of this series were obtained from one batch of well-mixed juice and are directly comparable. The juice was flash-heated to 89°C.(192.2°F.) and cooled before treatment. It was desirable to use unfiltered juice as a reference point in determining the effect of filtration on the viscosity of the juice. For this reference point a sample of the flash-heated juice was centrifuged to remove insoluble particles of sufficient size to interfere with the flow of juice through the Ostwald pipette. Juice centrifuged in a Sharples supercentrifuge at approximately 9,500 times the force of gravity was not clear but did not deposit a sediment on standing for periods of over six months and gave reproducible viscosity values.

Filtration through Seitz filter sheets was accomplished by means of a silver-plated Seitz laboratory filter, size No. 14, using a single filter sheet and exposing a section of 122.7 square centimeters to the flow of juice. Seitz K2, K3, K5, K7, GP, S, and sterilizing EK filter sheets were used in this study. The juice was forced through the filters by air pressure maintained between 15 and 16 pounds per square inch except in the case of the more porous sheets where the initial speed of flow was so great that the pressure could not be maintained in the system. In these cases the pressure dropped to as low as 12 pounds per square inch until the filters became sufficiently clogged that a pressure of 15 pounds could be maintained. The first 25 ml. of juice to flow through each sheet was discarded since it, in many cases, contained small bits of loose fiber which were washed from the surface of the sheet. Thereafter the juice was collected in consecutive units of 110 ml. each. In cases where very rapid initial flow was obtained two or more of these 110-ml. units were combined to form a single sample. The time required for each unit volume to flow through the sheet was recorded. The samples were sealed in four-ounce bottles, pasteurized by heating to 88°C.(190.4°F.) in a boiling water bath, and labeled according to the grade of filter sheet through which they passed and their sequence in passing through the sheet. These samples were observed for clarity and analyzed to determine their viscosity and content of alcohol precipitate and pectic acid.

Juices F2 and F3 were prepared from Winesap apples in the same manner as F1 but were not flash-heated. Juice F2 was clarified by adding a commercial, soluble, pectin-decomposing enzyme preparation at the rate of one pound per 100 gallons of juice and holding at room temperature for 18 hours. A portion of this clarified juice was filtered through a porous Seitz K3 sheet and a duplicate portion through an EK sterilizing sheet. Juice F3 was clarified by means of gelatin and tannin, according to the method given by Carpenter and Walsh (1932). After settling for 12 hours duplicate portions of this juice were filtered through Seitz EK and K2 sheets. A complete clarification was obtained by both processes. These filtrations were made using suction and a Büchner funnel. The flow

of these clarified juices was not affected by clogging during the course of the filtration. Samples were bottled and pasteurized by the method described for Sample F1.

Apple juice F4 was pressed from Winesap apples, flash-heated to 89°C., and cooled. Part of this juice was filtered through a Seitz EK filter sheet using a Büchner funnel and suction. The remainder was filtered through a Whatman No. 2 filter paper. Apple juice F5 was prepared from Winesap apples and was filtered without a preliminary heat treatment. Samples from Lot F4 were bottled and pasteurized, by the method described for previous samples, before analysis, whereas samples from Lot F5 were analyzed without pasteurization.

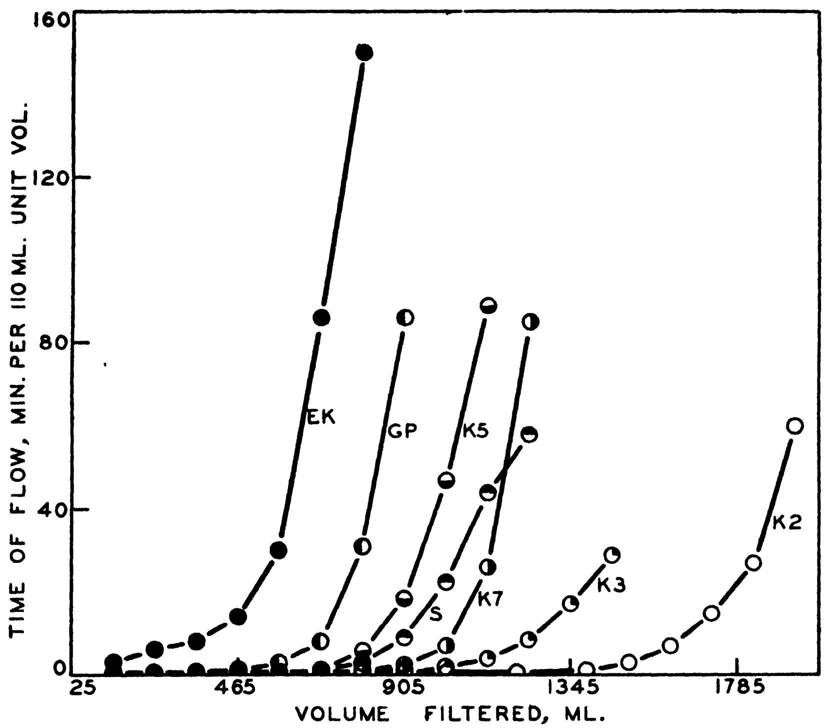

FIG. 1. Relation of time of flow per unit volume to the number of milliliters filtered for various grades of Seitz filter sheets.

The relative clarity of Juice F1 for each successive 110-ml. unit volume filtered, as determined by both transmitted and reflected light, for each of the filter sheets studied is shown (Table 1). The time required for each successive unit volume to pass through each of the filters in relation to the volume filtered at the end of each unit volume is shown (Fig. 1).

Data on the viscosity and content of alcohol precipitate and pectic acid of each unit volume of juice filtered appear (Fig. 2). The determination of viscosity was believed to be a more sensitive index of changes in the juice than the determination of alcohol-precipitate fractions and for this reason the latter determinations were omitted in some cases when the

TABLE 1

Clarity of Juice Fl Filtered Through Seitz Filter Sheets[1]

Filter sheet	Light[2]	Successive 110-ml. unit volumes filtered																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
K2	T	+ ++	+ ++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+
	R	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++	+ ++	+ ++	+ ++	+ ++	++
K3	T	+	+	—	—	—	—	—	—	—	—	—	—	—	—	....	....	....
	R	++ ++	++ ++	++ ++	++ ++	++ ++	+ ++	+ ++	++	++	++	+	+	+	+	....	....	....
K5	T	—	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....	....
	R	++	++	++	++	++	+	+	+	—	—	....	....	....	....	....	....	....
K7	T	—	—	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....
	R	+	+	+	+	+	+	+	—	—	—	—	....	....	....	....	....	....
GP	T	—	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....
	R	+	+	+	+	+	+	+	—	....	....	....	....	....	....	....	....	....
S	T	—	—	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....
	R	—	—	—	—	—	—	—	—	—	—	—	....	....	....	....	....	....
EK	T	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....
	R	—	—	—	—	—	—	—	....	....	....	....	....	....	....	....	....	....

[1] — = Clear juice; + = very faint turbidity; ++ = noticeable turbidity; +
++ = medium turbidity; ++
++ = quite turbid.

[2] T = transmitted light; R = reflected light.

change in viscosity was found to be small. Where analyses were omitted the points on each side of the omitted value were connected by a broken line. To facilitate comparison straight, horizontal lines were drawn through this figure to show the corresponding values for Juice F1 which had been submitted to a centrifugal force 9,500 times gravity.

Results on the effect of filtration on clarified Juices F2 and F3, data on the analysis of Juice F1 which had been centrifuged but not filtered, and also data on the analysis of Juices F4 and F5 are presented (Table 2). The results of ash analysis of the Seitz filter sheets are shown (Table 3).

TABLE 2

Effect of Filtration and Centrifuging on Apple Juice

Juice No.	Treatment	Description of juice	Viscosity centipoise	Alcohol precipitate	Pectic acid	Total astringency (as tannin)
				pct.	*pct.*	*pct.*
F1	Submitted to centrifugal force of 9,500 G.	Very cloudy. Did not settle on standing.	1.745	.054	.018	.068
F2	Enzyme clarified. Filtered through K3 sheet.	Clear	1.494	.019	.007	.061
F2	Enzyme clarified. Filtered through EK sheet.	Clear	1.492	.018	.005	.059
F3	Gel-tan clarified. Filtered through K2 sheet.	Very slight turbidity.	1.655	.043	.013	.047
F3	Gel-tan clarified. Filtered through EK sheet.	Clear	1.682	.043	.012	.047
F4	Flash-heated to 89°C. and cooled. Filtered through Whatman No. 2 filter paper.	Cloudy	2.071	.082	.034	......
F4	Flash-heated to 89°C. and cooled. Filtered through EK sheet.	Clear	1.834	.047	.017	......
F5	Unclarified. Filtered through Whatman No. 2 filter paper.	Cloudy	1.951	.085	.043	......
F5	Unclarified. Filtered through EK sheet.	Clear	1.819	.050	.021	......

TABLE 3

Ash Content of Unused Seitz Filter Sheets

Sheet grade No.	Ash	Sheet grade No.	Ash
	pct.		*pct.*
K2	5.3	GP	18.5
K3	11.1	S	20.8
K5	12.8	EK	29.1
K7	16.8		......

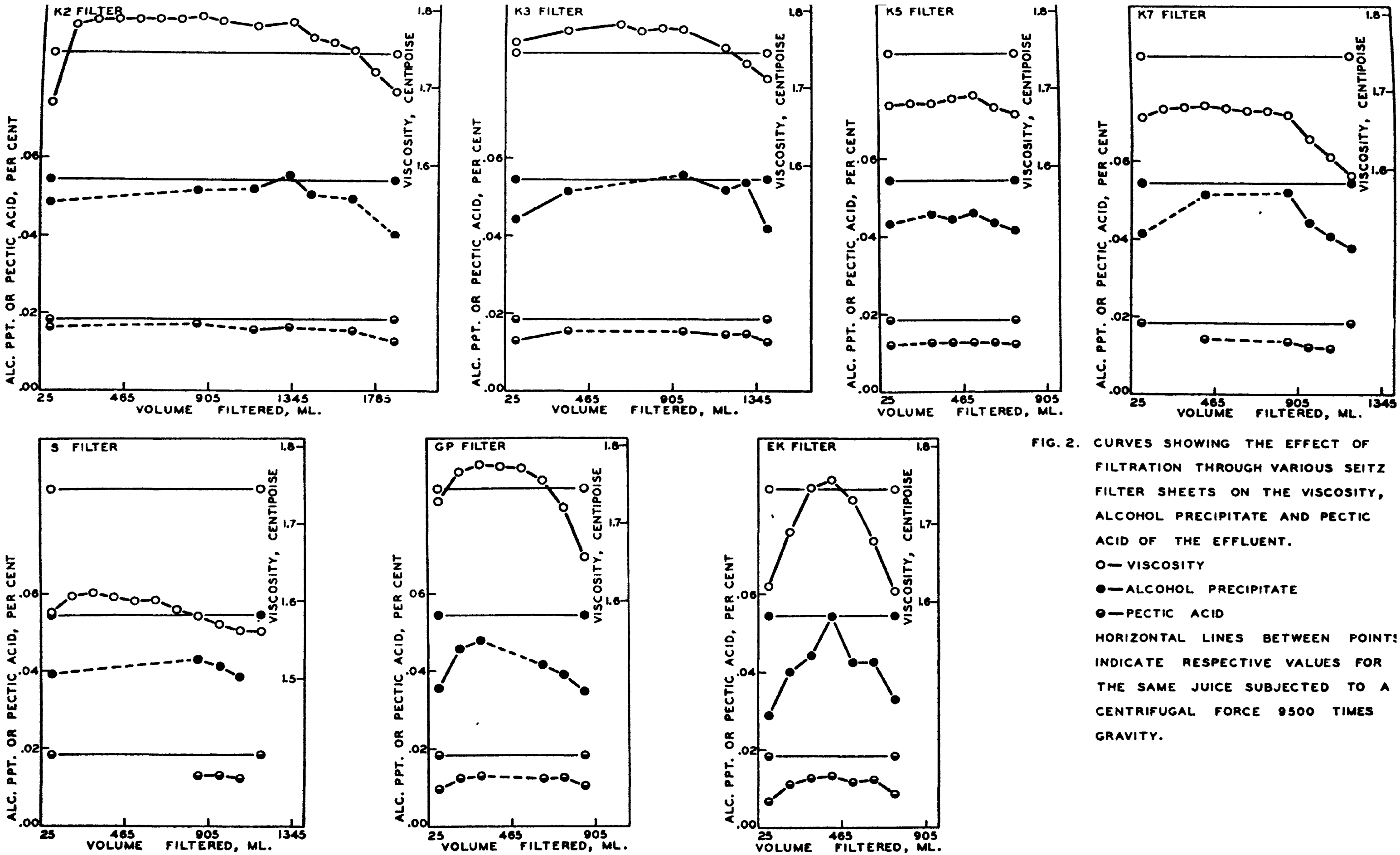

FIG. 2. CURVES SHOWING THE EFFECT OF FILTRATION THROUGH VARIOUS SEITZ FILTER SHEETS ON THE VISCOSITY, ALCOHOL PRECIPITATE AND PECTIC ACID OF THE EFFLUENT.

O— VISCOSITY

●—ALCOHOL PRECIPITATE

⊖—PECTIC ACID

HORIZONTAL LINES BETWEEN POINTS INDICATE RESPECTIVE VALUES FOR THE SAME JUICE SUBJECTED TO A CENTRIFUGAL FORCE 9500 TIMES GRAVITY.

DISCUSSION OF RESULTS

Only the EK and S filter sheets yielded a juice clear in both transmitted and reflected light (Table 1). Juice filtered through the GP, K7, and K5 was clear in transmitted light but varied from quite turbid to clear by reflected light. All but the first two unit volumes of juice to pass through the K3 sheet were clear in transmitted light but no clear juice, either by transmitted or reflected light, was obtained by filtration through the K2 sheet. Cloudy juices were found to decrease in turbidity as more juice was passed through the filter. This decrease in turbidity was accompanied by a decrease in speed of flow of the juice through the filter sheet owing to clogging (Fig. 1). The speed of flow of juice through the various sheets and the rate of clogging were not, in every instance, related to the clarity of the juice. Thus the S sheet permitted a greater speed of flow and did not clog as rapidly as the EK, GP, or K5 sheets but yielded a less turbid juice than either the GP or K5 sheet. The K7 sheet yielded a less turbid juice than the K5 and permitted more juice to pass before clogging became effective in reducing the speed of flow. All sheets on becoming clogged assumed nearly the same speed of flow.

These results are not to be interpreted as the exact response which is to be expected from a given grade of filter sheet, as the sheets of a given grade undoubtedly vary in porosity from lot to lot and apple juice itself is not constant in composition or properties. The results do indicate relative differences that may be expected among the various grades of filter sheets.

There did not appear to be a noticeable difference in color between juices obtained by the various filtration processes as has been reported by previous workers. However, observations on the alcohol precipitate and pectic-acid fractions during the course of analysis showed that those juices which had been filtered through the more porous sheets before they became clogged yielded a precipitate darker in color than did juice filtered through EK, S, and GP sheets.

The viscosity of juice filtered through Seitz filter sheets varied not only with the grade of the sheet but also during the course of the filtration through the sheet. These changes in viscosity as a result of filtration followed a general trend (Fig. 2) and were accompanied by corresponding changes in the alcohol precipitate and pectic-acid content of the juice. The first unit volume of juice to flow through any sheet was found to be lower in viscosity than unit volumes immediately following. Thus from a relatively great loss in viscosity in the first juice to pass through the filter the loss became gradually less in succeeding unit volumes until the juice retained a maximum viscosity. Thereafter the loss in viscosity gradually increased as more juice passed through the sheets.

The form of the viscosity, alcohol-precipitate, and pectic-acid curves suggests the possibility that viscosity-contributing substances present in apple juice were adsorbed by the filter sheets. The effect of this adsorption on the viscosity gradually decreased as the adsorbing surfaces became saturated, whereupon the juice retained a maximum viscosity. The higher viscosity of the second and third unit volume of juice filtered was not accompanied by a marked change in speed of flow. As shown (Fig. 1) all filter sheets but the EK passed four or more unit volumes at a rate of one

unit volume a minute or less before clogging became effective in slowing down the flow. The different Seitz filter sheets did not exhibit equal adsorbing ability as shown by the rise in the viscosity and composition curves during the early phase of the filtration. The EK sterilizing sheet and the GP and K2 sheets exhibited the greatest adsorbing power. Only slight rises in the viscosity and composition curves were obtained in the case of the Seitz S, K2, K3, K5, and K7 sheets, although the S sheet reduced the viscosity of the juice more than any other sheet.

Ash analyses were made on unused sheets to determine whether or not this apparent adsorbing power was related to the mineral or organic constituents of the sheets. These data (Table 3) show no relation between the adsorbing power of the sheets and their ash content. The EK sterilizing sheet exhibited the greatest adsorbing power and possessed the highest ash content but the K2 sheet also exhibited considerable adsorbing power and had the lowest ash content. The K3, K5, and K7 sheets exhibited only slight adsorbing power but varied in ash content. It was not possible to estimate the adsorbing power of the S sheet because the reduction of viscosity by filtration masked any possible adsorption which occurred.

After the maximum values for viscosity and composition were reached these values were again decreased by the filter. The break from the maxima for each of the sheets occurred at approximately the point in the filtration where clogging became effective in reducing the speed of flow of the juice (Fig. 1). This indicates that when clogging slowed down the flow of juice the pore size had been reduced to such a point that viscosity-contributing substances were removed by sieve action. The extent of this removal was roughly proportional to the degree of clogging of the filter.

Using the maxima of the viscosity curves as an index, the Seitz sheets may be placed in the following order of decreasing porosity: K2, K3, GP, EK, K5, K7, and S. On the basis of data presented (Fig. 1) on the speed of flow of juice through the filter, the order of decreasing porosity is K2, K3, K7, S, K5, GP, and EK. It is thus evident that the ease with which juice passes through a filter is not necessarily an index of the amount of viscosity-contributing substances removed during its passage.

Unfiltered Juice F1 which had been centrifuged at 9,500 G., was lower in viscosity, as shown by the base lines (Fig. 2) than the more viscous unit volumes to pass through the Seitz EK, GP, K2, or K3 sheets, while the viscosity of juice filtered through K5, K7, and S sheets was consistently reduced below that of the centrifuged juice. From this data it is evident that the centrifuging treatment is effective in removing viscosity-contributing substances from apple juice and such juice cannot be considered as equivalent to freshly pressed juice in viscosity. It is therefore impossible to determine the exact loss in viscosity suffered by the juice as a result of filtration, and losses are only relative.

The pectic-acid content of filtered juice was found to be consistently lower than that of centrifuged juice. The unit volume of juice which retained the greatest proportion of its alcohol-precipitate fraction when passed through the EK sheet was equal in this fraction to the centrifuged juice, and the unit volume having the highest value for the K2 and K3 sheets was higher than centrifuged juices. From these results it appears

that contrifuging removed viscosity-contributing substances not removed by filtration while permitting the juice to retain greater proportions of the pectic-acid and alcohol-precipitate fractions. These results also show that the loss in viscosity is not due to a removal of the insoluble suspended matter present in the juice. Juice filtered through the K5 and K7 sheets was slightly turbid and centrifuged juice was very turbid although these juices were equal or lower in viscosity than the clear juice of maximum viscosity obtained from the EK sheet.

Special interest is attached to the behavior of the Seitz EK sterilizing filter sheet because of its ability to remove microorganisms in the course of filtration. Fig. 1 shows that this sheet was much slower in passing juice than any of the other sheets and clogged much more rapidly. It appears to have a greater adsorbing capacity than the other sheets studied since it was effective in reducing the viscosity of the first 355 ml. of juice to pass through it, whereas the GP sheet, next greatest in adsorbing capacity, appeared to adsorb viscosity-contributing substances only from the first 245 ml. of juice. From the maxima in the curves of juice filtered through the EK sheet it is apparent that the pore size was greater than for K5, K7, and S sheets and was sufficiently great to permit the passage of compounds found in the alcohol-precipitate fraction until clogging took place. This is in agreement with the experiments of Carpenter, Pederson, and Walsh (1932) in which it was found that the extreme dimensions of large yeast cells did not approach the magnitude of the diameter of the capillaries in the EK sheet. These authors found it impossible to explain the removal of organisms from a fluid by means of Seitz EK sheets as a sieve action.

The possibility that microorganisms are removed as a result of adsorption alone cannot be proposed as the mechanism by which EK sheets operate unless the sheets have considerably greater adsorbing power for such organisms than for viscosity-contributing substances, as these substances appear to be adsorbed only from the first juice to pass through the sheet. Carpenter, Pederson, and Walsh (1932) found most of the filtered organism in the entering surface of the sheet and the amount of juice which could be sterile-filtered seemed to be limited only by the clogging of the sheet.

The filtration process disturbed only slightly the alcohol-precipitate-pectic acid ratio. These factors appeared to be removed in amounts proportional to their concentration both by adsorption during the early part of the filtration and by sieve action when the filters had become clogged. No relationship was found between viscosity and the alcohol-precipitate or pectic-acid fractions. These results show that the viscosity-alcohol-precipitate relationship can be disturbed by such a simple physical treatment as filtration or centrifuging.

The EK sterilizing filter sheet appeared to have no more effect on the viscosity or the alcohol-precipitate, pectic-acid, and astringent fractions of juice clarified by enzyme or gelatin-tannin treatment than did the more porous K2 and K3 sheets (Table 2).

Juices F4 and F5, when filtered through EK sheets, contained about 58 per cent as much alcohol precipitate and 50 per cent as much pectic acid as duplicate juice filtered through Whatman No. 2 filter paper. The vis-

cosity of these juices was also reduced much more by the EK sheets than by the Whatman paper. The values for these factors in juice filtered through the EK sheets represents more nearly the lower sections of the respective curves (Fig. 2), because a comparatively large volume of juice was passed through the sheets after clogging had reduced the speed of flow. Clogging probably acted to reduce these values in juice filtered through the Whatman filter paper although adsorption, if effective at all in Whatman paper, would be only slight, since the mass of the paper is much less than that of Seitz sheets. These results show that Seitz EK sheets do not reduce juice to a constant value for viscosity and composition but merely tend to reduce these values. Thus a juice originally high in viscosity might conceivably possess a higher viscosity after filtration through an EK sheet than unfiltered juice which was very low in viscosity when pressed.

Results here presented must be considered as examples of the extreme in rate and degree of clogging attained in filtration processes. Filter presses most commonly employed in commercial practice are constructed so as to permit the use of filtration aid, such as diatomaceous earth. These filter aids serve to remove from the juice those constituents which cause clogging before they reach the filter sheet, thus permitting a greater volume of juice to pass through the filter at viscosities corresponding to the maxima in the curves presented here. These results emphasize the value of avoiding a clogged condition of the filter if a maximum body is to be retained.

With the recent emphasis placed on "body" in apple juice it appears desirable to give more attention to the variety and condition of the fruit used in preparing the juice. Should a high "body" prove essential to quality in apple juice it would prove very simple to fortify this property by adding either a pectin extract prepared from apples or commercial pectin to the juice after filtration. This would permit the production of a highly clarified juice possessing a "body" found to be optimum for best quality. Juices have been prepared in this laboratory having a consistency similar to a heavy sirup by the simple addition of pectin.

SUMMARY

The effect of filtration on the appearance, viscosity, and alcohol-precipitate and pectic-acid fractions of apple juice was studied. Raw unheated juice, flash-heated and cooled juice, and juices clarified by enzyme treatment and by gelatin-tannin fining were investigated. Seven grades of Seitz filter sheets and Whatman No. 2 filter paper were used as filtering mediums. Changes caused by filtration were compared with those caused by centrifuging juice.

Filtration reduced the viscosity and the alcohol-precipitate and pectic-acid content of juice. The extent of these effects depended upon the type of filter medium used and upon the degree of clogging of the filter but was not related to the porosity of the filter as determined by the speed of flow or by the clarity of the filtered juice.

In addition to removal of viscosity-contributing colloidal fractions by sieve action Seitz sheets appeared capable of adsorbing these materials from the juice. This adsorption capacity was most pronounced in the EK sheet but did not appear to be related to the ash content of the filter sheets.

The alcohol precipitate-pectic acid ratio was disturbed only slightly by filtration but these fractions were not an accurate index of viscosity.

Centrifugal force was as effective in reducing the viscosity and the alcohol-precipitate fraction as filtration through Seitz K2, K3, GP, or EK sheets before they became clogged, although the centrifuged juice was more turbid.

Enzyme-clarified and gelatin-tannin fined juice clogged Seitz EK, K2, and K3 sheets very slowly. The EK sheets had no more effect on the viscosity, alcohol-precipitate, pectic-acid, and astringent fractions of these clarified juices than did the K2 or K3 sheets.

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SIGNIFICANCE OF ADEQUATE CONTROLS IN ABSOLUTE STERILITY DETERMINATIONS

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During the last eight years the use of paper containers for the distribution of milk has increased rapidly, and with this growth of a new industry considerable literature has developed on the sterility of these containers. The bulk of the literature reports that between 70 and 80 per cent of containers were sterile when tested by a modified rinse test similar to that given in the 1941 edition of *Standard Methods for the Examination of Dairy Products.* This method consists of adding 20 c.c. of sterile water to the container to rinse the inside and plating half or 10 c.c. of the rinse water on three plates. It differs from the rinse method outlined in previous editions of *Standard Methods,* which specified a 100-c.c. rinse and the plating of one c.c. In testing containers in which seldom more than two or three organisms are present, it was felt that this small subdivision of the rinse water for the plate sample permitted too great an opportunity for error in estimating the number of organisms present and, consequently, would lead to inaccurate data in reference to the absolute sterility of the container.

Three and one-half years of routine bacteriological examinations of prefabricated fiber milk containers, together with supplementary investigations, indicate that the 70 to 80 per cent sterility reported in the literature is too low, and that the containers are nearly 100 per cent sterile. These experimental data evaluate the unavoidable technique contamination factor. An application of this factor to the routine values cited in the literature accounts for the discrepancy noted above.

A summary of the routine testing for three and one-half years (Table 1) shows that of 30,718 containers tested by the rinse test, 78.5 per cent were found to be free of any bacteria or, in the terminology used in this paper, showed 78.5 per cent sterility. The non-sterile containers contained an average of less than .003 of the limit established by the U. S. Public Health Service Milk Ordinance and Code which allows one organism for each milliliter of capacity.

INTRACONTAINER TEST

The 10 c.c. of rinse water remaining in the container after the 10-c.c. rinse test sample had been withdrawn were purposely left in each of 23,786 containers for the intracontainer test. In this test, agar is added to the 10 c.c. of water remaining in the container after 10 c.c. were removed for plating in the rinse test. The agar is allowed to solidify in the container and the container is incubated 48 hours at 37°C.(98°F.). This test showed 87.8 per cent sterility, or 9.3 per cent higher sterility than the rinse test on the same containers, indicating that the difference in the sterility values

of the two tests was due to external or technique contamination occurring during the manipulation of the plates of the rinse test.

ROUTINE CONCURRENT STERILE GLASS RINSE TEST CONTROLS

As a complete control on the routine rinse testing of the daily container production, a considerable number of rinse tests was made concurrently on sterile glass containers. During a three-year period of manufacturing control testing, 10,542 sterile 500-c.c. Erlenmeyer flasks were rinsed, along with the containers of the rinse test, to serve as controls of the standard method. This procedure showed only 81.5 per cent sterility in these flasks which had been sterilized for 30 minutes in steam at 121°C.(249.8°F.). In other words, external or technique contamination accounted for 18.5 per cent non-sterility. This high incidence of external contamination appears to imply faulty and careless technique. However, it is merely an indication that in testing for absolute sterility it is necessary to use adequate multiple controls. The most accurate data would be obtained by using one control for each container tested, but practical experience has shown that one control for each three containers gives a fairly accurate picture of external contamination and reduces the amount of work and materials required. This conclusion is based on the fact that the contamination found in rinsing 10,542 sterile flasks was 18.5 per cent, against a contamination of 21.5 per cent on 30,718 containers. Applying this control correction, the sterility value would be 97 per cent instead of 78.5 per cent, as reported for the rinse test.

TABLE 1

Summary of Results of Routine Bacteriological Examinations of Quart Fiber Milk Containers

Year	Rinse test			Sterility test		
	Number tested	Number sterile	Sterile	Number tested	Number sterile	Sterile
			pct.			pct.
1939	1,085	640	59.0	1,326	889	67.0
1940	9,362	7,675	81.9	9,461	7,910	83.6
1941	12,519	9,800	78.2	12,600	9,114	72.3
1942 through July	7,752	6,028	77.7	7,829	5,694	72.7
Total	30,718	24,143	78.5	31,216	23,607	75.6

Year	Intracontainer test			Routine concurrent sterile glass rinse test controls		
	Number tested	Number sterile	Sterile	Number tested	Number sterile	Sterile
			pct.			pct.
1939	....	....	.....	....	....	.....
1940	4,317	3,979	92.1	2,570	2,192	85.3
1941	11,717	10,171	86.8	4,382	3,579	81.6
1942 through July	7,752	6,738	86.9	3,590	2,825	78.7
Total	23,786	20,888	87.8	10,542	8,596	81.5

When plating milk, which always has a fairly large number of bacteria present, the presence of one or two colonies on the plate which originated from external or technique contamination are of little significance. However, when plating to determine the absolute sterility of canned foods,

sutures, milk and other perishable food containers, etc., one or two contaminating colonies are of great significance, and the use of adequate controls is necessary if the data obtained is to be accurate.

Mudge and Foord (1940) called attention to the use of multiple controls when testing for absolute sterility and reported as high as 56 per cent of their controls contaminated under the laboratory conditions in which they were working. Williams and Clark (1942), of the National Canners Association Laboratory in Washington, recently reported an examination of 111 cans of salmon. Salmon is given a very long process at rather high temperatures, not only to produce a sterile product but also to soften the bones of the fish. It is extremely unlikely that any organisms could survive the process. They did their plating in a small inoculating room, ventilated with filtered air, and used extreme precautions to eliminate external contamination; they reported as high as 45 per cent contamination, yet the salmon revealed no abnormalities in appearance, flavor, or odor. These authors stressed the importance of using multiple controls in tests for absolute sterility.

STERILITY TEST

A routine sterility test is also made to check the rinse test. In this test, nutrient broth is added directly to the test container. The container is thoroughly shaken and incubated for 48 hours at 37°C.(98°F.). At the end of the incubation period, the broth is inspected for the presence or absence of bacterial growth. Of 31,216 containers tested, 75.6 per cent of the containers were sterile. This test showed a lower incidence of sterility than either of the other two tests, even though the method of performing the test was such that there is less opportunity for external contamination to affect the results. A study of this apparent discrepancy is included in the supplementary investigations described below.

SUPPLEMENTARY INVESTIGATIONS

During the period from January 22 to May 16, 1942, three consecutive containers were taken from a production line at hourly intervals and were coded "A," "B," and "C." The routine rinse and intracontainer tests were performed on containers coded "A." The routine regular sterility test was performed on the containers coded "B." The containers coded "C" were treated as follows:

Forty c.c. of nutrient broth were added to the container and the container was thoroughly shaken to rinse the inside, after which it was stored for 48 hours at the legal milk storage temperatures (40 to 50°F.). At the end of the storage period, the container was again shaken to rinse the inside and to distribute evenly any organisms that might be present in the broth. A drop of alcohol was placed on a panel of the container about one inch from the bottom. A sterile hypodermic needle attached to a sterile syringe was used to puncture the wall of the container through the drop of alcohol, and 20 c.c. of the broth in the container were removed and transferred to a sterile cotton-stoppered culture tube. This tube was then incubated for 48 hours at 37°C.(98°F.). The results of this test are recorded in Table 2, under the heading "syringe technique sterility test." A drop of molten paraffin was placed on the hole in the container made by the hypodermic needle puncture to seal the remaining 20 c.c. of broth in the container, after which the container was incubated 48 hours at 37°C. (98°F.). The results of this test are recorded in Table 2 under the heading, "cold hold sterility test."

Broth was added to sterile, cotton-stoppered culture tubes for approximately every third container tested in the regular sterility test and served as controls for these tests as well as for the pouring of the broth into the containers for the syringe technique sterility test.

Syringe technique sterility test controls were prepared as follows:

One culture tube containing sterile nutrient broth was exposed to the air for every three containers tested. These tubes were exposed to the air for the same amount of time as the tubes which received the broth from the syringe.

The air of the laboratory was sampled by means of a Wells Air Centrifuge while the above tests were being performed.

Following the incubation period, the containers, tubes, or plates of each of the tests were inspected for the presence or absence of bacterial growth and the amount of growth was noted. The growth in all cases was subjected to microscopic examination and identification after gram staining. Results of these tests are shown (Table 2).

A study of the sterility data from the regular sterility test ("B") and from the syringe technique sterility test ("C_1") shows that the incubation of broth in the container at 37°C.(98°F.) by the regular sterility test procedure yields an untrue low sterility value with reference to the percentage of containers which would contribute organisms to milk under commercial conditions. This is proved by the fact that only 72.4 per cent of the 1,000 containers in the regular sterility test were sterile, whereas, in the syringe technique sterility test more than 93 per cent were sterile.

It is obvious that the 37°C.(98°F.) incubation of the broth in the container causes some penetration of the broth through the waterproofing surface which does not occur when the container is subjected to the maximum of 10°C.(50°F.) temperature storage condition, which is normal for milk. In other words, this test subjects the container to an abnormal temperature storage condition which it is not required to meet in normal commercial use.

Since the technique contamination in adding the broth to the containers was 5.8 per cent, one-half of this contamination, or 2.9 per cent applies to the half portion of broth which was finally removed for the syringe technique sterility test ("C_1"). This amount added to the direct technique contamination which is shown by the controls on syringe transfer to the tubes (three per cent) gives a total of 5.9 per cent technique contamination applicable to the syringe technique sterility test samples. As the total non-sterile samples in this test amount to 6.6 per cent, 6.6 — 5.9 = .7 per cent non-sterile after allowance for technique contamination. This value (.7 per cent) represents half of the total non-sterile containers, since only half of the broth originally introduced into the containers was used in the syringe technique. Therefore, the actual value for non-sterile containers in this test is 1.4 per cent, or a total of 98.6 per cent sterile.

The incidence of sterility in the rinse test and sterile glass rinse test controls (rinse of sterile 500-c.c. flasks) was approximately the same. If allowance is made for the technique contamination indicated by the rinse controls, the rinse test ("A") would show a sterility value of 99.7 per cent, which is in good agreement with the 98.6 per cent found in the syringe technique sterility test ("C_1").

TABLE 2

Results of Supplementary Investigations on Quart Fiber Milk Containers

Container code	Sterility data			Bacterial distribution							
	Number tested	Number sterile	Sterile	Total number	Average per non-sterile container	Gram-positive rods	Staphylococci	Large cocci	Mold	Actinomyces	Others
			pct.			pct.	pct.	pct.	pct.	pct.	pct.
Rinse test: A	1,000	787	78.7	630	2.96	79.0	12.4	3.8	2.9	1.9	.....
Sterile glass rinse test controls: —	333	263	79.0	208	2.97	81.7	9.6	2.9	1.0	4.8	.....
Intracontainer test: A_1	1,000	867	86.7	282	2.12	94.3	0.7	......	3.6	1.4	.....
Regular sterility test: B	1,000	724	72.4	......	......	98.6	0.35	0.7	0.35	.....	.....
Cold hold sterility test: C	1,000	630	63.0	......	......	99.0	0.5	......	0.5	.....	.....
Syringe technique sterility test: C_1	1,000	934	93.4	......	......	89.5	4.5	1.5	3.0	.....	1.5
Sterility test controls: —	359	338	94.2	......	......	95.2	4.8	......	.....	.....	.....
Syringe technique sterility test controls: —	310	301	97.0	......	......	77.8	11.1	11.1	.....	.....	.....
Air samples (one test each day): —	57	3	5.3	446	......	77.2	12.8	5.6	0.9	3.1	0.4

The intracontainer test ("A_1") showed a higher percentage of sterility than the rinse test ("A"), undoubtedly owing to the fact that the performance of the test entails much less manipulation and fewer chances for external contamination. The effect of the incubation temperature on the paraffin coating is not as noticeable in the intracontainer test as in the regular sterility test ("B"), because there is little free moisture in the solid agar to penetrate into the paper.

The distribution of the various types of contaminating organisms throughout the various tests is of interest and shows quite clearly that much or most of the contamination of the rinse test is external contamination. A comparison of the rinse test and air sample bacterial distribution shows them to be about the same, whereas the types of bacteria found in the cold hold sterility test ("C") were 99 per cent gram-positive rods. The rinse test, sterile glass rinse test controls, syringe technique sterility test controls, and air samples all show approximately the same percentages of each of the bacterial types, indicating that the bacteria contaminating these tests were undoubtedly air-borne contaminants. On the other hand, the distribution of the bacterial types in the sterility tests, the performance of which allows for a very small chance for external contaminations, showed that 98.6 to 99 per cent of the contaminating organisms were gram-positive rods or the sporeforming types which are picked out of the paper by the broth during the incubation.

The syringe technique sterility test tubes, when contaminated, showed a significant percentage of staphylococci to indicate some air-borne contamination, which fact again lends weight to the assumption that the per cent contamination of the syringe technique sterility test controls may be subtracted from the per cent contamination of the syringe technique sterility test tubes to give an absolute sterility value of 98.6 per cent to the containers.

An additional test was made to study the effect of the 37°C.(98°F.) incubation temperature on the paraffined surface of the containers of the regular sterility test. Containers were fabricated from paper which had an average bacterial count of 850 organisms per gram and also from paper with an average count of 14 organisms per gram. The following data were obtained from testing these containers:

Paper bacterial count	Containers tested	Rinse test		Intracontainer		Sterility		Syringe technique sterility	
		Number sterile	Sterile	Number sterile	Sterile	Number sterile	Sterile	Number sterile	Sterile
			pct		pct		pct.		pct.
14	102	82	80.4	93	91.1	81	79.4	98	96.0
850	204	165	80.8	128	62.8	53	26.0	193	94.6

Whereas the incidence of sterility as determined by the rinse tests of both lots of containers was about the same, the sterility test showed a much lower sterility value with the high count paper than with the low. The syringe technique sterility test, which measures more accurately the actual number of containers contributing bacteria to milk under the conditions to be found in commercial use, showed approximately the same

incidence of sterility in both lots. These data furnish substantial additional evidence that the sterility test, as outlined in the latest *Standard Methods for the Examination of Dairy Products*, is inaccurate as a measure of sterility because the conditions of the test are more severe than those required of the container in normal legal use. Consequently, the sterility values resulting from this test are much too low.

SUMMARY

A summary of the data accumulated during three and one-half years of daily routine bacteriological control testing of Canco prefabricated fiber milk containers shows that 78.5 per cent of 30,718 containers tested by the standard rinse test were sterile. If a correction for technique error is made, 97 per cent of the containers were sterile. The 21.5 per cent containers rated as unsterile by the standard rinse test contained less than three organisms per container, or approximately .003 of the limit established by the U. S. Public Health Service Milk Ordinance and Code.

Data are presented which show that when testing containers by the sterility test as outlined in the *Standard Methods for the Examination of Dairy Products*, an inaccurate measure of the number of containers which would contribute organisms to the milk under commercial conditions is obtained, owing to the fact that the incubation of broth at 37°C.(98°F.) in the container subjects the container to conditions which it is not required to meet in commercial use. Correcting for this and technique errors, 98.6 per cent of the containers were sterile, which is in good agreement with the rinse test data.

Control data for the rinse test show that when testing for absolute sterility of any type container, at least one control should be made for every three containers tested in order to accurately interpret the results of the tests.

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VITAMIN A ACTIVITY OF LEAN MEAT AND FAT FROM CATTLE FED VARIOUS LEVELS OF CAROTENE

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Very few studies have been made of fat (adipose) and lean tissues of beef carcasses as a source of vitamin A-active compounds. This is due largely to relatively low potency of these foods as compared with liver, butter fat, or green leafy vegetables. Isolated values for vitamin A content of beef tissues, however, are given in the literature. Booher and Marsh (1941) listed beef suet as containing 600 International Units per 100 grams but found no vitamin A in lean beef: Munsell (1940) reported 50 International Units per 100 grams for lean beef. Guilbert and Hart (1934) suggested low vitamin A activity of beef fat from vitamin A-deficient cattle when they described the tissue taken from such animals as pure white in color.

Fraps and co-workers (1937), Leuschen and co-workers (1937), and others have shown that vitamin A activity of butter fat is dependent largely on carotene and related pigments supplied to cattle in feeds, such as green pasture, hays, silages, and yellow corn. Guilbert and Hart (1934) studied vitamin A storage in cattle livers but the conditions which might affect vitamin A content of edible meat and fat are not as well known.

Several lean and fat samples from cattle fed rations of varying carotene content have been available to this laboratory during the past three years. The object of this paper is to report results of vitamin A assays and some carotene analyses on these tissues.

MATERIALS AND METHODS

Samples From Pasture and Dry Lot-Fattened Cattle: This group of samples represents a comparison of the vitamin A activity of beef fat from cattle approximately three and one-half years of age which had been fattened on pasture as compared with grain and mixed hay fed in dry lot. The animals were fed at the Virginia Agricultural Experiment Station in co-operation with the Bureau of Animal Industry.

In these experiments 40 steers approximately two and one-half years of age were selected each year and divided into two equal groups. Group 1 was turned on bluegrass pasture with salt as the only supplement. The second group was fed in dry lot a diet of corn, cottonseed meal, mixed hay, and salt. Both groups were maintained at approximately the same degree of fatness. A more detailed description of the plan of feeding these cattle has been given by Hankins and Barbella (1941), while the findings on carotene and vitamin content of the meat tissue have been summarized in annual reports of the Chief of the Bureau of Animal Industry by Mohler (1939, 1940, and 1941).

After completing their feeding period, 10 cattle from each group were shipped to the U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md., for slaughter and grading. Raw fat samples for assay were taken from the chilled, dressed carcasses one week after the animals were killed. Exterior fat was removed over the 12th rib from the left side of each carcass. Individual samples ranged from 100 to 150 grams in weight. They were composited by lots, ground and mixed thoroughly, packed in half-pint fruit jars, and stored in a refrigerator at —17.8°C. (0°F.).

Approximately 10 days after slaughter, the 12th rib cuts from the right sides of the carcasses were roasted in ovens maintained at a temperature of 150°C.(302°F.). The cuts were allowed to come to an internal temperature of 70°C.(158°F.) before removal from the ovens; then they were chilled in a refrigerator and exterior fat was removed. Care was taken to collect the same areas of fat as used in the case of the raw cut. The samples were composited and processed as in the case of the raw fat samples. Thus there were four samples, viz., raw and cooked fat from both grass-fed and grain-fed cattle. Samples of this kind were taken from the Virginia cattle in 1940 and 1941. Spectrophotometric determinations for carotene. also, were made on extracts of the 1940 samples.

Samples From Beef Cattle Fed Controlled Levels of Carotene: These assay samples were taken from cattle fed at the Texas Experiment Station, Spur, Texas, in co-operation with the Bureau of Animal Industry. These experiments were planned to determine the vitamin A requirements of fattening beef cattle. A detailed description of the animals and plan of the work has been reported by Jones and co-workers (1938) and Riggs (1940). In general, the calves used were depleted of vitamin A reserves, as indicated by night blindness, and subsequently fed definite levels of alfalfa-leaf meal of known carotene content. The three lots selected for this assay were fed daily 1,250, 2,500, and 5,000 micrograms of carotene in alfalfa-leaf meal per 100 pounds of body weight.

The samples consisted of the 12th rib cuts which were removed from the carcasses after slaughter of the cattle at Fort Worth, Texas. The ribs were shipped in a refrigerated car to the Beltsville Research Center and arrived 12 days after slaughter. The full-length ribs were cut back to 61 per cent length and trimmed to approximate retail style. Each rib was separated into external fat covering, lean and intramuscular fat, and bone. The bone was discarded and the other samples were ground, packed in fruit jars, and stored at 0°F.

Beef Tissues From Vitamin A-Deficient Cow: This animal had been maintained on a ration low in carotene for three years. She received a basal diet of the following percentage composition: beet pulp, 45; white corn, 40; linseed meal, 7; soybean meal, 7; and salt and bonemeal, each .5. In addition 1,200 grams of oat straw and a low level of alfalfa-leaf meal were fed. At the time of slaughter the animal was fat and had extensive anasarca or generalized edema or swelling of the legs, brisket, and shoulders which is apparently due to vitamin A deficiency. The fat and lean tissues from this animal were soft and watery owing to the extensive edema.

The sample for vitamin A assay was taken from the eighth to 12th ribs and was divided, as in the case of the other samples, into external fat and lean with intramuscular fat.

Sample of Tissues From Dry-Lot Steers: This group of animals consisted of four steers which were used in record-of-performance studies at Beltsville. The steers were weaned when they weighed 500 pounds. Before weaning they were on pasture and were fed a grain supplement consisting of corn, 4 parts by weight; oats, 3; wheat bran, 2; and linseed meal, 1. Between weights of 500 to 900 pounds, pasture feeding was discontinued and the grains fed remained the same but the mixture was changed to the proportions of 5:2:1:1. In addition the steers received salt and U. S. No. 2 leafy alfalfa hay. The animals were apparently normal in every respect when slaughtered at 900 pounds live weight. As in the case of the Texas cattle the vitamin A-assay samples consisted of two composites: (1) the outside fat covering of the 12th rib cuts and (2) the lean and intramuscular fat from the same ribs.

Biological Assays: The rats used for biological assay were 25-day-old albino males and females. They were housed in conventional screen-bottom cages kept in rooms with controlled temperature and humidity. With slight modifications the basal diet and assay procedure were the same as those described in the second supplement to U. S. Pharmacopoeia XI.

Jars of assay samples were removed as needed from the 0°F. refrigerator room, kept at temperatures below —6.7°C.(20°F.) during the assay period, and were weighed out in a room at 1.1°C.(34°F.). Each jar usually provided material for a two to three weeks' feeding period. Weighed portions of the beef tissues were fed to the rats every two days over the six-week feeding period. The amount of supplement fed, on a daily basis, ranged from 400 micrograms for the fat to four grams for the lean samples. Consumption of the supplements was usually complete within a few minutes except in a few cases where the rats were on very low levels of vitamin A. Crystalline carotene, dissolved in peanut oil and checked for purity and concentration by means of the spectrophotometer, was used as the standard of reference. This was fed from a calibrated syringe directly into the animal's mouth.

EXPERIMENTAL RESULTS

Results of biological assays of the beef samples which have been described are given (Table 1).

Spectrophotometric analyses for carotene on composite fat samples from the 1940 Virginia beef cattle showed the fat from pasture-fed animals contained 103.9 micrograms of carotene per 100 grams. When roasted this decreased to 62.8 micrograms. Fat from the lot-fed animals had a carotene content of 50.5 micrograms per 100 grams when analyzed raw and 44.9 micrograms when roasted. Absorption characteristics of the carotene in extracts from fat of the grass-fed cattle were definitely superior to those of the grain-fed and hay-fed animals. Very poor spectrographic-absorption curves were obtained from the carotene-containing extracts of roasted fat from both groups, which indicates that considerable oxidative destruction had taken place. The limited results obtained were fully in agreement with the biological assays, however, but it is evident that the vitamin A activity of fats is largely accounted for by preformed vitamin A. The fat from the

grass-fattened cattle was more yellow than that from the grain- and hay-fattened animals.

DISCUSSION

The data show that the vitamin A activity of beef tissues, like butter fat, is dependent on the carotene intake of cattle (Table 1). For example,

TABLE 1

Vitamin A Content of Tissues From Beef Cattle

Origin of samples	Diet of cattle	Daily level of carotene	Tissue [1]			Vitamin A in tissues
			Kind	Content of lean [2]	Condition	
		μgm./cwt.		*pct.*		*I.U./100 gm.*
Virginia, 1940	Pasture	High	Adipose	0	Raw	660
Virginia, 1940	Pasture	High	Adipose	0	Roasted	540
Virginia, 1940	Grain-mixed hay	Moderate	Adipose	0	Raw	420
Virginia, 1940	Grain-mixed hay	Moderate	Adipose	0	Roasted	270
Virginia, 1941	Pasture	High	Adipose	0	Raw	600
Virginia, 1941	Pasture	High	Adipose	0	Roasted	450
Virginia, 1941	Grain-mixed hay	Moderate	Adipose	0	Raw	450
Virginia, 1941	Grain-mixed hay	Moderate	Adipose	0	Roasted	335
Texas	Grain-silage-alfalfa	1,250	Lean	79.3	Raw	14
Texas	Grain-silage-alfalfa	2,500	Lean	78.5	Raw	16
Texas	Grain-silage-alfalfa	5,000	Lean	78.1	Raw	33
Texas	Grain-silage-alfalfa	1,250	Adipose	0	Raw	33
Texas	Grain-silage-alfalfa	2,500	Adipose	0	Raw	33
Texas	Grain-silage-alfalfa	5,000	Adipose	0	Raw	96
Beltsville, Md., vitamin-A-deficient cow	Grain-straw-alfalfa	1,360	Lean	62.8	Raw	38
Beltsville, Md., vitamin A-deficient cow	Grain-straw-alfalfa	1,360	Adipose	0	Raw	54
Beltsville, Md., steers	Grain-alfalfa	Low	Lean	75.3	Raw	29
Beltsville, Md., steers	Grain-alfalfa	Low	Adipose	0	Raw	57

[1] All tissues taken from rib cut. The adipose tissue consisted of the external covering of fat while the lean consisted of the muscles and intramuscular fat exclusive of external fat covering.
[2] Figures in this column obtained by subtracting per cent ether extract (chemical fat) from 100.

fat from the Texas beef cattle maintained on a carotene level of 1,250 micrograms of carotene per 100 pounds of body weight shows a vitamin A content of 33 International Units of vitamin A per 100 grams. When the carotene intake was raised to a 5,000-microgram level, the vtiamin A of the beef fat was increased to 96 International Units per 100 grams. This conclusion is supported by other data in the table including the figures showing high vitamin A content of the fat from animals that received a high level of carotene from pasture.

The lack of significant differences in the vitamin A content of tissues from cattle on the 1,250- and 2,500-microgram levels, the vitamin A-deficient cow, and the dry-lot-fed steers probably indicates that these animals were all receiving an inadequate amount of carotene for appreciable vitamin A storage in the tissues.

Other experiments with cattle have shown that 5,000 micrograms per hundred pounds of carotene are more than sufficient for apparently normal reproduction, Davis and Madsen (1941), and to prevent night blindness, Schmidt (1941). The relatively low vitamin A content of 96 International Units per 100 grams of fat from the beef cattle fed a 5,000-microgram level of carotene, however, shows that this amount in the ration is not sufficient to produce maximum storage of vitamin A in beef tissues.

Roasting beef fat under the conditions described above destroys some of the vitamin A activity. This is shown to be the case in all of the raw and cooked fat comparisons as indicated by a loss of biological activity and poorer absorption characteristics of the carotene extracts. This is in agreement with what is generally well known in regard to cooking vitamin A-containing foods.

The data are probably not extensive enough to show the maximum vitamin A content obtainable in beef tissue. The fat from the grass-fed Virginia beef cattle is probably representative of beef fats of maximum vitamin A content. Additional information is needed on the vitamin A content in the lean from such animals. Beef of relatively high vitamin A content can also be expected from grain-fed cattle receiving hay which is rich in carotene. This is indicated by the assay showing 420 International Units per 100 grams in fat from 1940 Virginia grain- and hay-fed animals. The lean and fat from dry-lot-fattened cattle receiving grain and restricted amounts of carotene below 5,000 micrograms per 100 pounds were found to be as low in vitamin A as the tissues from a cow showing symptoms of vitamin A deficiency.

SUMMARY

Results of biological assays for vitamin A content of edible fat and lean from beef cattle fed controlled rations, varying widely in carotene content, are reported. Values for biologically-active vitamin A in beef fat ranged from 660 International Units per 100 grams for pasture-fed cattle to 33 International Units for cattle fed a relatively low carotene level. Roasting beef fat destroys some of its potential vitamin A activity. Carotene intake of cattle is a controlling factor in the vitamin A content of edible beef cuts, including both fat and lean portions.

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FACTORS INFLUENCING TEXTURE OF PEAS PRESERVED BY FREEZING. II[1]

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It has been shown by the writers (1942) that (1) when frozen peas have tough skins after boiling for five minutes the skin texture usually is not improved by boiling for an extra ten minutes; (2) the skins and cotyledons of cooked frozen peas are toughened by vining, the commercial method of separating peas from the vines and pods, but that the skins only are toughened when there is a delay between the time of vining and freezing; (3) washing peas immediately after vining and keeping them cool between vining and freezing decreases but does not prevent toughening of the skins; and (4) there is wide variation in skin texture of peas of a given size and variety grown in the same field but harvested on different dates.

The purpose of this paper is to present the results of a study of the effects of additional handling procedures on the texture of skins of cooked frozen peas, as follows: (1) skin texture variations for peas separated by brine flotation after scalding; (2) comparison of the skin texture of fresh peas, peas held at —17.8°C.(0°F.) for 24 hours, and peas held at the same temperature for six months; (3) comparison of the effect of delay between vining and freezing and between vining and cooking of fresh peas; (4) the effect of thawing and refreezing peas prior to cooking; and (5) the separate effects of bruising and vine juice on the skin texture of cooked frozen peas.

EXPERIMENTAL PROCEDURE

The methods employed in growing, harvesting, and handling the peas were the same as those described in paper No. I by the writers (1942) up

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to the stage of packaging and freezing the peas. For this investigation, as soon as the peas were scalded, cooled, and drained, they were packaged in 12-ounce waxed cartons, wrapped in moisture-vapor-proof cellophane, and heat-sealed. The packages were placed in a freezer at approximately —17.8°C.(0°F.) and were stored at this temperature until the measurements of texture were made. Unless exception is noted the peas were placed in the freezer within two hours after vining. Any special handling procedures for particular studies are described in the study to which they apply. After the storage periods, the peas were sized and cooked as described in Paper No. I. The texture measurements of skins of the peas were made with motor-operated apparatus No. 2 described by Candee and Boggs (1941). In the investigation in which cotyledon texture was measured, apparatus No. 1 described by the same writers was employed. The skin texture value was the average load in kilograms required to penetrate three layers of skins with a one-eighth-inch, steel, ball-bearing penetration point, and the crushing value for the cotyledons was the average load required to crush one cotyledon to one-fourth of its thickness. One hundred measurements of cotyledons for all sizes of peas and one hundred measurements of skins of frozen peas of Sizes 5, 6, and 7 were made; but only 50 measurements of skins were made on frozen peas of Sizes 3 and 4 and on fresh peas of any size, because the variation in readings for the latter were relatively small.

The standard error of the mean penetration or crushing value and the standard error of differences of means were calculated according to Snedecor (1938). In all calculations of significant differences of means, one more digit was used for both the mean and the standard error of the mean than is reported in the tables of results. The standard error of the mean gives information regarding how close a second mean value is expected to be to the one reported if the study were repeated under the same conditions. A highly significant difference means the chances are 99 in 100 that the difference is a real one, and a significant difference means that the chances are 95 in 100 that the difference is a real one.

RELATIONSHIP OF TASTE SCORES TO MECHANICAL MEASUREMENTS OF TEXTURE

Mechanical measurements of texture of foods are of limited value unless they are related to tasters' judgments of quality. The description of the quality as determined by tasters for a given range of crushing values, which were previously reported by the writers (1942), are again used here. Because some changes were made in the penetration apparatus the study of the relationship of taste scores to penetration values was repeated. The scoring group consisted of the writers and one or two other research workers, all of whom had had much experience in scoring the quality of frozen peas. Scores of nine, eight, and seven indicate that the scorers considered the skins of these peas of high market quality; scores of six, five, and four were for skins considered marketable but tougher than those receiving scores of seven and higher; skins of peas receiving scores of three, two, and one were considered too tough to be marketed. Because it is more convenient in reporting, the three qualities will be referred to as Grades A, B,

and C throughout the remainder of this paper. The whole pea was eaten but only the skins were scored. The average of the scores by all the tasters is reported.

Two groups of peas were included in the taste study: (1) Thomas Laxton and Tall Alderman peas of varying maturity, which were handled and frozen by the usual procedure, and (2) Dark-Podded Thomas Laxton peas, different lots of which were treated individually as follows: vined, hand-podded, and then artificially bruised without the presence of vine and leaf juice; hand-podded, unbruised peas to which vine and leaf juice was added; and hand-podded, bruised, and vine juice added.

The average taste scores and penetration values (Table 1) indicate that skins of peas with penetration values of 1.00 or less were Grade A, those with values between 1.01 and 1.49 were Grade B, and those with values of 1.50 or higher were Grade C. Some of the pairs of taste scores and penetration values do not fit this classification, and the dividing points are not intended as final judgment but they should serve as guides to quality and assist the reader in interpreting the results reported in this paper. If the penetration values and taste scores for the four groups of peas are considered separately, the number of inconsistencies is small, especially for each of the maturity series. Most of the inconsistencies occurred either when the skin texture was border-line between the marketable and unmarketable conditions or when the cotyledons were unusually soft. Apparently if skins of peas from the two lots have the same penetration value but one has soft cotyledons and the other has hard ones, the skins of the lot with soft cotyledons seem worse by contrast of the two textures.

RESULTS AND DISCUSSION

Skin-Texture Variation for Peas Separated by Brines After Scalding: Brine separation, also called quality separation, has been widely used by the canning industry to separate overmature peas from a field-run lot and also to separate field-run lots into grade groups before canning them. Martin and Brotherton (1933), Strasburger (1935), Bonney and Rowe (1936), Jodidi (1937), Walls and Hunter (1938), and Burton (1938) have discussed the basis for specific gravity testing of canning peas, the precautions to be observed in order to obtain the most consistent separation, and the effectiveness of the method, especially the correlation of brine separation with federal grades for canned peas. Campbell and Diehl (1940) and Parker (1940) have discussed some problems encountered with brine separation of freezing peas. Burton (1938) discussed the effect of scalding prior to brine separation and he also discussed the desirability of eliminating certain sieve sizes of peas before the test is made.

Brine flotation of thawed, frozen peas from which the skins have been removed is the basis of texture rating in the tentative United States standards for grades of frozen peas developed by the United States Department of Agriculture (1939). Campbell and Diehl (1940) and Muenchow (1940) have reported on the relationship of the tenderometer reading of fresh peas to the federal grade for the frozen peas which is obtained by brine flotation. Lee (1941) has reported on the relation of the specific gravity of thawed, frozen peas, obtained by determining the difference between

TABLE 1

Relationship of Taste Scores to Penetration Values of Skins of Cooked, Frozen Peas

Variety[1]	Tenderometer reading of field-run sample	Treatment[2]	Pea size	Average taste score[3]	Penetration value
					kg.
T. Lax	129	Vined	7	1.3	1.95
T. Lax	115	Vined	6	1.3	1.65
T. Lax	129	Vined	6	1.8	1.70
T. Ald	116	Vined	6	1.8	1.60
T. Ald	119	Vined	6	2.0	1.48
D. P. T. Lax	95	H.p., v.j.	6	2.3	1.43
D. P. T. Lax	118	H.p., v.j.	5	2.3	1.41
D. P. T. Lax	118	H.p., v.j.	6	2.3	1.34
D. P. T. Lax	118	H.p., br., v.j.	6	2.5	1.76
D. P. T. Lax	118	Vined	6	2.5	1.40
T. Lax	129	Vined	5	2.5	1.53
T. Lax	111	Vined	6	2.5	1.63
D. P. T. Lax	118	H.p.	6	2.7	1.27
T. Ald	109	Vined	6	2.8	1.24
D. P. T. Lax	118	H.p., br.	6	2.9	1.55
T. Ald	119	Vined	5	3.0	1.13
D. P. T. Lax	95	Vined	5	3.2	1.48
T. Ald	116	Vined	5	3.3	1.25
T. Lax	99	Vined	6	3.3	1.65
D. P. T. Lax	95	H.p.	6	3.4	1.13
D. P. T. Lax	95	H.p., v.j.	5	3.4	1.31
D. P. T. Lax	118	H.p., br., v.j.	5	3.4	1.56
D. P. T. Lax	118	H.p., br.	5	3.4	1.59
T. Lax	115	Vined	5	3.5	1.51
D. P. T. Lax	118	H.p.	5	3.6	1.27
D. P. T. Lax	118	Vined	6	3.6	1.44
D. P. T. Lax	95	H.p., br.	6	3.8	1.60
T. Ald	109	Vined	5	3.8	0.96
T. Lax	95	Vined	6	3.8	1.28
D. P. T. Lax	95	H.p., br.	5	4.1	1.31
T. Lax	99	Vined	5	4.5	1.28
D. P. T. Lax	95	H.p.	5	4.7	0.93
T. Lax	111	Vined	5	4.8	1.40
T. Lax	95	Vined	5	5.0	1.02
T. Ald	94	Vined	6	5.3	1.31
T. Lax	95	Vined	4	7.0	0.89
T. Ald	94	Vined	5	7.5	1.04
T. Ald	109	Vined	4	7.8	0.76
T. Ald	93	Vined	4	8.5	0.80

[1] T. Lax.—Thomas Laxton; T. Ald. Tall Alderman; D.P.T.—Dark-Podded Thomas Laxton. [2]See pages 512 to 514 for details of the following special treatments: h.p.—hand podded; v.j.—vine and leaf juice added; br.—bruised. [3] Nine was best possible score; one was poorest score.

the weight of the sample in air and the weight in a mixture of xylene and carbon tetrachloride, the specific gravity of which is 1.00, to organoleptic tests, to the brine test included as a part of the federal grades for frozen peas, and to the alcohol-insoluble solids content of frozen peas.

Brine flotation, then may be carried out at two stages and the purposes of the two are entirely different. Brine flotation as used in the federal

grades for frozen peas and the specific gravity method of Lee are intended to evaluate the quality of frozen peas after they are packed and ready for the market; the purpose of brine flotation or quality grading of peas before they are frozen is to separate field-run material into different grades prior to freezing and packing.

The brine separation study reported here is part of a larger investigation being conducted by the Western Regional Research Laboratory, U. S. Bureau of Agricultural and Industrial Chemistry. The skin-texture measurements of the peas separated by brines of different densities were intended to supplement the information obtained in the larger research plan. Because the personnel of the co-operating agencies is changing, the skin-texture results are being reported at this time. The purpose of this investigation was to determine whether brine separation of scalded peas is a satisfactory means of separating them into skin-texture groups.

Two varieties of peas, Tall Alderman and Thomas Laxton, were included in the study. Each variety was harvested on several dates. For each day's harvest, after the peas were vined, cleaned, washed, scalded, and cooled, a large lot of the peas was placed in six-per cent salt brine; the floaters were removed, washed, and frozen, and the sinkers were entered into an eight-per cent brine. This procedure was repeated in 10-, 12-, 14-, and 16-per cent brines, and each lot of floaters was washed and frozen. Both the floaters and sinkers in the 16-per cent brine were retained. After approximately four months of storage, the Size 5 peas of each brine sample were graded out of the field-run sample, cooked, and the penetration values of the skins determined on the peas of this one size for all lots (Table 2).

For peas of a given variety harvested on a given date there was a tendency (Table 2) for peas which floated in increasingly dense salt brines to have increasingly tough skins. If the penetration values for skins of peas harvested on different dates are compared, however, it is clear that the brines did not satisfactorily separate peas into skin-texture groups. It may also be noted that, for peas with a given tenderometer reading and separated with a given brine, the Thomas Laxton variety of peas had very much tougher skins than did the Tall Alderman variety. The large differences in skin texture of peas from any one brine are especially surprising when it is recalled that all of the values in Table 2 are for Size 5 only, and field-run lots of peas of these varieties at prime maturity contain many larger peas. It may be concluded that unless the maturity of the samples is controlled brine flotation of scalded peas is of little value in separating peas into skin-texture groups.

Texture Changes After 24 Hours and Six Months of Freezing Storage: This investigation was undertaken in order to try to explain increasing tenderometer readings with increasing periods of freezing storage of peas, an observation which has been reported by one of the commercial packers in Washington. Dark-podded Thomas Laxton peas at two stages of maturity were used in this study. They were handled in the usual manner until the peas were scalded and cooled. When the peas had been cooled to 18°C.(64.4°F.), the tenderometer reading was taken and one lot of fresh peas was cooked in the same manner as previously described for frozen peas and the penetration value for the skins was determined. Or-

dinarily a somewhat longer cooking period is used for fresh peas than the six minutes used here for frozen peas, but it seemed undesirable to introduce a cooking variation in this investigation. The remainder of the peas were frozen in the usual manner. After the peas were in the freezer for 24 hours, when they were solidly frozen, some of them were removed from storage. One lot was thawed to 18°C.(64.4°F.) and the tenderometer reading was taken. The others were cooked and the skin texture was measured in the usual manner. After six months of storage the remainder of

TABLE 2

Skin-Texture Variation for Peas Separated by Brines After Scalding

Variety	Date of harvest	Tenderometer reading of field-run lot of fresh, unscalded peas	Penetration values for skins of Size 5 peas						
			Float in 6% brine	Float in 8% brine	Float in 10% brine	Float in 12% brine	Float in 14% brine	Float in 16% brine	Sink in 16% brine
	1940		kg.	kg.	kg.	kg.	kg.	kg.	kg.
Tall Alderman...	7/3	93	0.47	0.41	0.70	0.85	......	......	......
Tall Alderman...	7/5	108	0.48	0.47	0.70	1.09	1.12	1.44	......
Tall Alderman...	7/6	120	0.86	0.83	0.83	1.00	1.15	1.60	2.27
Tall Alderman...	7/8	141	1.34	1.26	1.26	1.71	2.02	2.39	2.42
Thomas Laxton........	6/24	......	0.87	0.81	0.87	0.98	......	......	......
Thomas Laxton........	6/25	......	1.06	0.75	0.99	0.94	1.29	......	......
Thomas Laxton........	6/26	99	......	0.91	1.03	1.43	1.53	......	......
Thomas Laxton........	6/27	108	......	1.29	1.24	1.67	1.51	......	......
Thomas Laxton........	6/28	112	......	1.35	1.44	1.76	1.63	2.79	......
Thomas Laxton........	6/29	115	......	1.56	1.25	1.54	1.83	......	......
Thomas Laxton........	7/1	125	......	1.33	1.15	1.20	1.81	1.89	2.17

the peas were removed, and the tenderometer reading and skin-texture values were determined in the usual manner. For the more mature sample, both the tenderometer reading and the penetration values were for Size 6 peas only; the results are summarized (Table 3).

The skins of the peas held in the freezer for 24 hours had practically the same penetration values as the fresh peas and in no case was the difference significant (Table 3). After six months of freezing storage, however, there was a large increase in the penetration values; the difference for both maturity lots was highly significant. On the basis of taste results, the skins of the more mature sample, the 499 series, had changed from excellent quality to unmarketable material during the six months of storage.

In contrast to the results for skin texture, the crushing values show that the cotyledons toughened considerably during the first 24 hours in the freezer; the difference for each of the comparisons was highly significant. The effect of freezing storage after the first 24 hours and up to six months is not clear. The crushing value for the cotyledons of the less mature sample did not change significantly but that for the more mature sample decreased significantly. Cotyledons with crushing values of .78 or less are considered of excellent quality, those with values between .79

TABLE 3

Texture of Fresh Peas and Peas Held in the Freezer for 24 Hours and for Six Months

(Dark-Podded Thomas Laxton Variety)

Sample No.	Handling procedure prior to cooking	Average penetration value of skins		Average crushing value of cotyledons	
		Size 4	Size 6	Size 5	Size 6
		kg	*kg.*	*kg*	*kg*
477 a	Fresh	.63±.01[1]	0.77±.01	.80±.02	0.93±.03
477 b	—17.8°C. storage for 24 hr.	.64±.01	0.77±.01	.93±.02	1.19±.04
477	—17.8°C. storage for 6 mo.	.88±.02	1.04±.02	.98±.02	1.18±.04
499 a	Fresh	....	0.76±.01	....	1.28±.07
499 b	—17.8°C. storage for 24 hr.	....	0.78±.01	....	1.52±.08
499	—17.8°C. storage for 6 mo.	. ...	1.58±.03	....	1.28±.05

TENDEROMETER READING

Sample No.	Pea sizes	Handling procedure	Tenderometer reading
			lb./sq in
......	Field-run	Fresh, unscalded	95
477 a	Field-run	Fresh, scalded, cooled	67
477 b	Field-run	—17.8°C. storage 24 hr., thawed	86
477	Field-run	—17.8°C. storage 6 mo., thawed	112
......	Field-run	Fresh, unscalded	118
499 a	6	Fresh, scalded, cooled	78
499 b	6	—17.8°C. storage 24 hr., thawed	100
499	6	—17.8°C. storage 6 mo., thawed	112

[1]± refers to standard error.

and 1.13 are marketable but not excellent, and those with values above 1.19 are considered unmarketable. Thus the increase in crushing value of cotyledons during the first 24 hours in the freezer represents a rather serious texture change. The tenderometer readings of the samples increased a great deal after the peas were in the freezer for 24 hours, apparently owing to toughening of cotyledons, and they increased again after six months of storage, this increase apparently being due to toughening of skins. Prior to this study it had been thought that possibly thawed, frozen peas packed more closely than did fresh, scalded ones and that the packing might account for the increase in the tenderometer readings after the peas were frozen. Since this study, based on measurements of individual peas, shows that both cotyledons and skins toughen either during freezing or storage, it seems likely that the increased tenderometer readings were due, in part at least, to actual toughening of the individual peas.

Texture Changes With Varying Periods of Delay Between Harvesting and Freezing: As a continuation of the study on the effect of 24 hours and six months of storage on texture of peas, a second freezing investigation was undertaken in order to compare the effect of delay between vining and cooking of fresh peas with that for delay between vining and freezing followed by cooking after several months of storage of frozen peas. It was also the purpose to determine the comparative effects of freezing and of delay.

Tall Alderman peas harvested at four stages of maturity were used in this study. As soon as possible after vining, Lot 1 of each day's harvest was cleaned and scalded. This lot of peas was divided and one part was cooked immediately by the procedure previously described for frozen peas and the penetration measurements of the skins were made; the other part of Lot 1 was frozen by the usual procedure and after five months of storage the peas were cooked and the penetration values were determined. Following vining, Lot 2 was held at approximately 20°C.(68°F.) for four to six hours, then scalded and handled in the same manner as Lot 1. Because of the varying times of day at which peas were delivered from the field and the amount of time required to determine the penetration values of the fresh peas, it was not practical to keep the delay periods the same for the different days' harvests. The results of this investigation are presented (Table 4).

From the data presented (Part A, Table 4) it may be noted that the skins of all of the cooked, fresh peas, even the Size 7 of the overmature lots, that is the lots with tenderometer readings of 120 and 141, had penetration values below one kilogram, which places them in the Grade A classification on the basis of th^ taste scores. For the frozen peas, however, only the skins of Size 4 peas could be classified as Grade A and those of Sizes 6 and 7 were Grades B and C. Ten of the 16 samples of frozen peas of Sizes 6 and 7 had Grade C skins, that is the skins were considered too tough to be marketed although the skins of the same lots of peas before they were frozen were Grade A.

The penetration values for the skins of all lots and sizes of fresh peas varied only from .41 to .98 kilogram, while those for the same lots of frozen peas varied from .67 to 2.15 kilograms. Pea size, maturity, and delay, therefore, are shown to have more effect on skins of frozen peas than on skins of fresh peas.

The data (Part B, Table 4), which were derived from Part A, show that the increase in the delay period between harvesting and cooking unfrozen peas resulted in increases in penetration values for skins varying from .01 to .16 kilogram.

In the case of the frozen peas which were stored for five months at —17.8°C.(0°F.) prior to cooking and recording the penetration values, it may be noted from the data of Part B of Table 4, which were derived from Part A, that the increase in the delay period between harvesting and freezing resulted in an increase in penetration values for skins varying between .12 and .67 kilogram. It should be noted that the longer periods of delay resulted in only small increases in penetration values for Size 4 of the lots having tenderometer values of 93, 108, and 120, namely, .13, .16,

and .15 kilogram, respectively, and for Size 6 of the lots having tenderometer values of 93 and 108, namely, .12 and .19 kilogram. However, the differences in penetration values were in all cases highly significant.

From these results it would appear, then, that the differences in periods of delay between vining and cooking had only a small toughening effect on

TABLE 4

Effect of Delay Before Freezing on Skin Texture of Tall Alderman Peas of Varying Maturity

PART A

Date of harvest	Tenderometer reading of field-run sample	Delay between harvest and freeze	Penetration values					
			Size 4		Size 6		Size 7	
			Fresh[1]	Frozen[1]	Fresh	Frozen	Fresh	Frozen
1940	*lb./sq. in.*	*hr.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*
7/3	93	1	.41±.02	0.67±.02	.61±.01	1.15±.05	.69±.03	1.26±.03
7/3		4	.49±.02	0.80±.02	.67±.01	1.27±.03	.80±.01	1.53±.04
7/5	108	3	.47±.02	0.68±.02	.74±.01	1.36±.03	.87±.01	1.56±.04
7/5		6	.63±.01	0.84±.03	.82±.01	1.55±.04	.96±.02	1.94±.07
7/6	120	2	.49±.02	0.76±.03	.78±.02	1.44±.03	.95±.01	1.65±.07
7/6		6	.62±.02	0.91±.03	.83±.01	1.79±.05	.98±.01	1.97±.05
7/8	141	2	.60±.01	0.94±.03	.80±.01	1.52±.05	.91±.02	1.48±.03
7/8		6	.67±.02	1.33±.05	.82±.01	1.94±.06	.92±.01	2.15±.07

PART B

Increases in Penetration Values With Longer Delay Periods, Derived From Part A

Date of harvest	Fresh peas			Frozen peas		
	Size 4	Size 6	Size 7	Size 4	Size 6	Size 7
1940	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*
7/3	.08	.06	.11	.13	.12	.27
7/5	.16	.08	.09	.16	.19	.38
7/6	.13	.05	.03	.15	.35	.32
7/8	.07	.02	.01	.39	.42	.67

PART C

Increases in Penetration Values After Freezing, Derived From Part A

Date of harvest	Lots delayed shorter periods			Lots delayed longer periods		
	Size 4	Size 6	Size 7	Size 4	Size 6	Size 7
1940	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*	*kg.*
7/3	.26	.54	.63	.31	0.60	0.73
7/5	.21	.62	.69	.21	0.73	0.98
7/6	.27	.66	.70	.29	0.96	0.99
7/8	.34	.72	.57	.66	1.12	1.23

[1] Fresh means unfrozen, cooked; frozen means frozen and stored at —17.8°C.(0°F.) for five months, cooked.

the skins of fresh, unfrozen peas. It would also appear that the extent of toughening of skins of the frozen and stored peas resulting from the longer delay periods was associated with both the pea size and the maturity of the field-run samples. Small frozen peas, unless they were very mature, toughened to only a small extent, but most of the larger peas toughened

considerably with the longer periods of delay, and the more mature the lot the greater was the extent of the toughening of the skins.

The data (Part C, Table 4), which were derived from Part A, show that for a given lot of peas freezing plus the five months of storage at 0°F. resulted in larger increases in penetration values for skins than did the single factor of delay between vining and freezing. In every case the differences between the penetration values of the fresh and frozen samples were highly significant when the size of peas, maturity of the field-run lot, and periods of delay were the same. The increases following freezing were less for the Size 4 than for the larger peas and, in general, the more mature the field-run lot the larger were the increases in penetration values following freezing.

TABLE 5

Effect of Thawing and Refreezing Frozen Peas

Variety	Tenderometer reading of field-run sample	Handling conditions prior to cooking	Penetration values	
			Size 4	Size 6
	lb./sq. in.		*kg.*	*kg.*
Thomas Laxton	94	Unthawed	0.80±.03[1]	1.35±.04
Thomas Laxton	94	Thawed 24 hr. at 8°C. (46.4°F.)	0.81±.05	1.35±.03
Thomas Laxton	94	Thawed 7 hr. at 24.7°C. (76°F.)	0.85±.06	1.22±.02
Thomas Laxton	94	Thawed 24 hr. at 8°C., refrozen	1.11±.03	1.53±.02
Thomas Laxton	94	Thawed 7 hr., at 24.7°C., refrozen	1.12±.03	1.53±.04
Tall Alderman	108	Unthawed	0.81±.04	1.32±.02
Tall Alderman	108	Thawed 24 hr. at 8°C.	0.74±.03	1.34±.03
Tall Alderman	108	Thawed 7 hr. at 24.7°C.	0.76±.02	1.20±.02
Tall Alderman	108	Thawed 24 hr. at 8°C., refrozen	0.98±.03	1.60±.03
Tall Alderman	108	Thawed 7 hr., at 24.7°C., refrozen	1.06±.03	1.53±.03

[1]± refers to standard error of mean.

Texture Changes After Thawing and Refreezing: From the bacteriological standpoint the disadvantage of thawing and refreezing peas is recognized but whether thawing or thawing followed by refreezing of peas, within the limits required to prevent spoilage, is deleterious to quality has not been shown. A study was undertaken, therefore, to learn the effect of thawing and refreezing on one quality factor, skin texture.

Thomas Laxton and Tall Alderman peas whose tenderometer readings for a field-run sample were 94 and 108, respectively, were used in the investigation. The peas were handled and frozen in the usual manner. After approximately eight months of storage, one sample of each variety was cooked without preliminary thawing and the skin texture was measured. At the same time one lot of each variety was thawed in the package for 24 hours in an electric refrigerator maintained at approximately 8°C. (46.4°F.) and one lot was thawed for seven hours at approximately 24.7°C. (76°F.). At the end of the thawing period, half the thawed peas were cooked and the skin texture was measured, and the remainder of the thawed peas were returned to —17.8°C. (0°F.) storage for two weeks before they were cooked, without additional thawing, and the skins tested. The penetration results are summarized (Table 5).

Thawing peas for 24 hours at 8°C. had little effect on the penetration values of the skins of the peas and in no case was the difference significant Table 5). Considering the peas thawed for seven hours at 24.7°C., the penetration values for Size 4 were not significantly different from those for the peas which were not thawed before they were cooked, but Size 6 had significantly lower values. No explanation can be offered for this decrease in values. When the peas were refrozen following thawing at either temperature the penetration values of the skins of all of the peas increased significantly. Thawing, then, does not seem to toughen skins of peas, but thawing followed by refreezing seriously toughens them.

Separate Effects of Bruising and Vine Juice: The writers (1942) have previously reported that delay following vining seriously toughened the skins of peas preserved by freezing. Kohman and Sanborn (1936), reporting on canning peas, suggested that absorption of calcium from the vine and leaf juice during the delay following vining might account for the toughening of peas. The present investigation was undertaken in order to try to determine the effect of delay both when the peas had come in contact with vine juice and also when they had not had any vine juice on them but had been bruised. It was thought that if the factors causing toughening during vining were known, it might be possible to prevent at least some of the toughening. This would seem to be especially true if the vine juice were the cause because it might be possible to wash the peas immediately after they leave the viner, thus removing much of the vine juice.

Dark-podded Thomas Laxton peas harvested on two dates were used in the investigation. A field-run sample of one lot had a tenderometer reading of 94, and the other, 118. On each of the harvest dates, a plot of peas was mowed, and approximately one-fourth of the plants were vined. The pods of the remainder of the plants were harvested and podded by hand. For the first harvest date, the hand-podded peas were divided into three lots. One lot was untreated. Vine and leaf juice, obtained by grinding the plants in a food chopper and straining the pulp through cheesecloth, was thoroughly mixed with the second lot of hand-podded peas. The third lot was bruised in a miniature, motor-driven viner for one minute. On the second harvest date an additional lot of hand-podded peas was bruised and then the vine juice was added. This treatment was included in order to determine whether the combined effect of the artificial bruising and addition of vine juice caused the same amount of toughening as did vining under the usual commercial conditions. After the peas were vined or hand-podded and the various treatments following hand-podding were completed, each lot of peas was allowed to stand in a wooden box at approximately 21.1°C. (70°F.) for five hours. Such a period of delay between podding and freezing was considered typical for this area, where peas are usually vined on or near the pea fields and the vined peas are then transported to the freezing plant. Because of the time required for hand-podding there was an additional short period of delay in the pod for the hand-podded peas. A preliminary study had shown that such short periods of delay, if the peas were in the pods, resulted in little or no change in skin texture. At the end of the delay periods each lot of peas was thoroughly washed and then handled and frozen in the usual manner. After

approximately seven months of storage the peas were cooked and the skin texture was measured; the results are summarized (Table 6).

The commercially vined peas had higher penetration values than the hand-podded peas and, with the exception of Size 3 peas of Lot 1, the differences for each size for both lots were highly significant (Table 6). In the case of Size 3 peas of Lot 1 the difference was significant. The hand-podded peas which were bruised but did not come in contact with vine juice had higher values than peas which were hand-podded and received no additional treatment, and the difference for each size of the two lots was highly significant. The penetration values for the hand-podded peas to which vine juice was added were also higher than for hand-podded peas which received no additional treatment, and the difference for each

TABLE 6

Separate Effects of Bruising and Vine Juice on Skin Texture of Cooked, Frozen Peas of Dark-Podded Thomas Laxton Variety

Date of harvest	Tenderometer reading of field-run sample	Handling conditions prior to freezing	Penetration value			
			Size 3	Size 4	Size 5	Size 6
1940	*lb./sq. in.*		*kg.*	*kg.*	*kg.*	*kg.*
Lot 1						
5/17	94	Vine	0.92±.02[1]	1.18±.01	1.36±.02	1.42±.02
5/17	94	Hand pod only	0.61±.02	0.72±.02	0.93±.02	1.13±.02
5/17	94	Hand-pod, bruise	0.97±.02	1.25±.02	1.31±.02	1.60±.04
5/17	94	Hand pod, vine juice	0.80±.02	1.05±.02	1.31±.03	1.43±.02
Lot 2						
5/19	118	Vine	0.94±.04	1.11±.04	1.44±.03	1.40±.03
5/19	118	Hand-pod only	0.83±.03	0.98±.04	1.27±.03	1.27±.02
5/19	118	Hand-pod, bruise	1.06±.04	1.46±.03	1.59±.03	1.55±.03
5/19	118	Hand-pod, vine juice	0.99±.03	1.37±.04	1.41±.02	1.44±.03
5/19	118	Hand pod, bruise, vine juice	1.04±.03	1.33±.04	1.56±.03	1.76±.03

[1]± refers to standard error of mean.

size of the two varieties was highly significant. Thus bruising of hand-podded peas or the addition of vine juice to unbruised, hand-podded peas resulted in serious toughening of the skins of the frozen peas. Since both the contact of peas with the vine juice and the bruising of the peas caused toughening of skins, it would seem advisable to operate viners as slowly as practical at all times, and to further investigate the effectiveness of washing peas as soon as they are vined.

With the exception of Size 5 of Lot 1, all of the hand-podded peas which were bruised had higher penetration values than those for hand-podded peas to which vine juice was added, but the differences in penetration values were not significant for Sizes 3 and 4 of Lot 2. For the other sizes, namely, Sizes 3, 4, and 6 of Lot 1 and Sizes 5 and 6 of Lot 2, the differences were highly significant. Because the differences in penetration values for bruised peas and peas to which vine juice was added were not significant for all sizes, it cannot be said that one factor or the other definitely caused more toughening of skins, but since the bruised peas had

higher penetration values in seven of the eight comparisons and since the differences were highly significant in five of the eight comparisons, it would appear that bruising, to the extent used in this investigation, caused somewhat more toughening than the addition of vine juice. The peas in Lot 2, to which vine juice had been added following bruising, had higher penetration values than the commercially vined peas. The differences in penetration values for Sizes 4, 5, and 6 were highly significant and for Size 3 significant. Thus it is apparent that the artificial bruising and adding of vine juices was a more severe treatment than occurs in ordinary commercial vining. The main findings of the investigation are not altered, however, and if the toughening of skins which occurs during vining or during delay following vining is to be minimized, both the effects of the vine juice and the bruising of the peas must be taken into consideration.

SUMMARY

Varieties of peas suitable for freezing were grown under ordinary commercial conditions, vined, cleaned, scalded for one minute in water at 98.9°C.(210°F.), cooled in water to approximately 14°C.(57.2°F.), packaged in 12-ounce waxed-paper cartons, wrapped, and heat-sealed in moisture-vapor-proof cellophane, then frozen and stored at —17.8°C.(0°F.). After several months of storage the frozen peas were cooked without preliminary thawing in boiling water for six minutes, and the texture of the skins of all samples was determined by measuring the load in kilograms required to penetrate three layers of skins with a one-eighth-inch, steel, ball-bearing penetration point. In one study the texture of the cotyledons was also determined by measuring the load required to crush one cotyledon to one-fourth of its thickness. The special handling procedures required in order to investigate a particular treatment are apparent in the following summary of results:

1. For peas of a given variety, harvested from a given plot on the same day, there was a tendency for peas which were floated off from increasingly dense salt brines to have increasingly tough skins. However, if the peas of a given variety grown in a given field and separated by a given brine density were harvested on different days, that is, at different stages of maturity, the range of penetration values of a given size of peas was very wide. Brine separation, therefore, is not a practical means of separating peas into skin-texture groups.

2. Comparing cooked, fresh peas with peas held in the package at —17.8°C.(0°F.) for 24 hours and then cooked, the cotyledons of the frozen peas were tougher than those of the fresh peas but there was no difference in the skin texture.

3. Comparing peas held in the package at —17.8°C.(0°F.) for 24 hours with peas held at the same temperature for six months, the skins of the peas held for the longer period were considerably tougher than those held for only 24 hours. The results for the crushing value of cotyledons held for these periods were inconsistent and could not be interpreted.

4. Comparing the skin texture of vined peas held for one to three hours with similar peas held for four to six hours after vining before they were cooked, or frozen and stored for several months and then cooked, it was

found that the longer delay periods resulted in only a small toughening effect on the texture of the skins of all sizes of fresh peas investigated and for all stages of maturity investigated. For the frozen peas, however, the extent of toughening with the longer delay periods differed according to both the pea size and the maturity of the field-run sample. With the longer delay periods Size 4 frozen peas, unless they were from a very overmature, field-run lot, toughened no more than the fresh peas, but Sizes 6 and 7 from most of the maturity lots toughened a great deal and the more mature the field-run sample the more a given size of peas toughened.

5. Thawing peas for 24 hours at 8°C.(46.4°F.) or for seven hours at 24.7°C.(76°F.) prior to cooking did not toughen the skins of frozen peas, but if the peas, following thawing, were refrozen and stored for two weeks at —17.8°C.(0°F.) and then cooked without additional thawing the skins were seriously toughened.

6. Bruising of hand-podded peas which had not come in contact with vine juice and the addition of vine and leaf juice to unbruised, hand-podded peas each significantly toughened the skins of peas of Sizes 3, 4, 5, and 6. Bruising alone appeared to cause slightly more toughening than the addition of vine juice alone but the evidence was not conclusive.

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